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Review

Ether lipids

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ABSTRACT

The naturally occurring 1-*O*-alkyl-*sn*-glycerols and their methoxylated congeners, 1-*O*-(2′-methoxyalkyl)-*sn*-glycerols, are biologically active compounds, ubiquitously found in nature as diacyl glyceryl ether lipids and phosphoether lipids. The chief objective of this article is to provide a comprehensive and up to date review on such ether lipids. The occurrence and distribution of these compounds in nature are extensively reviewed, their chemical structure and molecular variety, their biosynthesis and chemical synthesis and, finally, their various biological effects are described and discussed. An unprecedented biosynthesis of the 2′-methoxylated alkylglycerols is proposed. The first synthesis of enantiopure (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol, the most prevalent 2′-methoxylated type alkylglycerol present in cartilaginous fish, is described. It was accomplished by a highly convergent five step process.

Contents

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1. Introduction

The chief objective of this article is to provide an up to date review on ether lipids that belong to the class of 1-*O*-alkyl-*sn*-glycerols. After a brief introduction the main emphasis is laid on 1-*O*-alkyl-*sn*-glycerols and their 1-*O*-(2′-methoxyalkyl)-*sn*-glycerol analogues. The occurrence and distribution of these compounds in nature, their chemical structure and molecular variety, their biosynthesis and chemical synthesis, and their various biological effects, are described, discussed and extensively reviewed. Finally, their well-known analogues, the platelet-activating factors (PAF) and the plasmalogens, are also briefly described and put into context with these ether lipids.

Ether lipids cover a great variety of lipid compounds carrying an ether linkage. Glycerol based ether lipids are normally minor constituents of most cell membranes in mammals, but in contrary, major components in archaeal cell membranes. Indeed, some fish of the Chondrichthyes class accumulate high amounts of ether lipids in their liver. The most prevalent glycerol ether backbones found in nature consist of an *O*-alkyl or *O*-alk-1′-enyl group attached to glycerol at position *sn*-1 (Fig. 1) (Mangold and Paltauf, 1983).

The absolute configuration of the chiral carbon atom located at the mid-position of the glycerol moiety is designated by the stereospecific numbering (*sn*). That terminology is based on the *pro-S* methylene carbon of the prochiral glycerol molecule being designated as the *sn*-1 position, the central carbon atom as the *sn*-2 position and the *pro-R* methylene group carbon as the *sn*-3 position. Accordingly, the naturally occurring 1-*O*-alkyl-*sn*-glycerol term implies that the ether alkyl chain is located at position *sn*-1 of glycerol (Garrett and Grisham, 1995).

1-*O*-Alkyl-*sn*-glycerols are highly bioactive compounds to which various therapeutic roles have been attributed (Iannitti and Palmieri, 2010). In non-polar lipid fractions of animals they occur as fatty acid diesters comprising two fatty acyl groups at their *sn*-2 and *sn*-3 positions. The 1-*O*-alkyl-2,3-diacyl-*sn*-glycerols are generally known as diacyl glyceryl ethers (DAGE). They also occur in phospholipid fractions as ether phospholipid derivatives comprising a fatty acid at the *sn*-2 position and a phosphodiester bonded polar head, usually ethanolamine or choline, at the *sn*-3 position of the glycerol moiety. The 1-*O*-alkyl-*sn*-glycerol based ether phospholipids are called plasmanyl-phospholipids and they include the PAFs, which are a group of lipid mediators with potent biological activities (Mangold and Paltauf, 1983; Mangold and Weber, 1987) (Fig. 2). The 1-*O*-(alk-1′-enyl)-*sn*-glycerol phospholipid analogues are essential constituents of animal lipid membranes and their 1-*O*-(alk-1′-enyl) diacylated analogues are usually called neutral plasmalogens. The ether phospholipids comprising 1-*O*-(alk-1′-enyl)-*sn*-glycerol ether backbones are called plasmenyl-phospholipids (plasmalogens) and are suggested, among other things, to be involved in the myelination process (Gorgas et al., 2006).

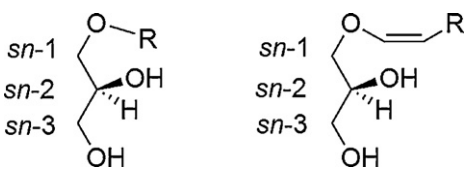


Fig. 1. 1-*O*-alkyl-*sn*-glycerol and 1-*O*-(alk-1′-enyl)-*sn*-glycerol.

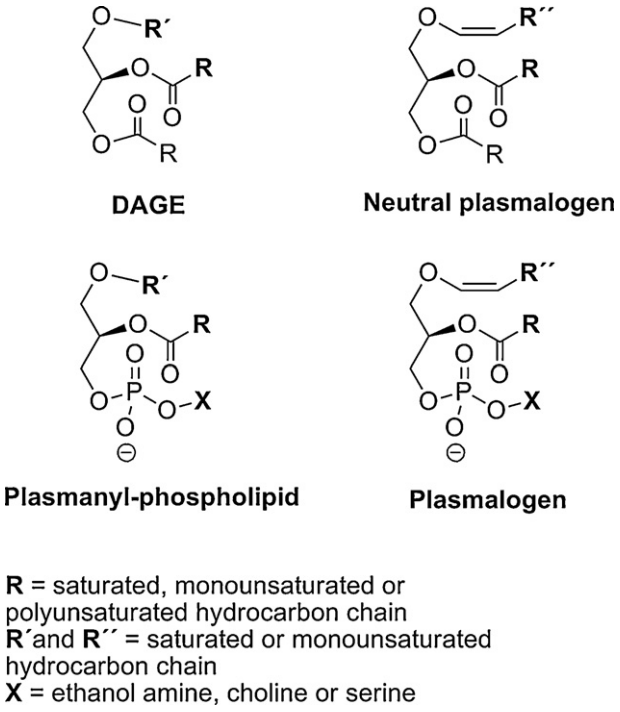


Fig. 2. 1-*O*-alkyl and 1-*O*-(alk-1′-enyl)-*sn*-glycerol based neutral and phosphorylated ether lipids.

The methoxylated alkylglycerols (2′*R*)-1-*O*-(2′-methoxyalkyl)-*sn*-glycerols are glycerol ethers of the *sn*-1 configuration. They possess a methoxyl group located at the 2-position of the 1-*O*-alkyl chain (Fig. 3). Like their unsubstituted analogues they occur as fatty acid diesters and alkyl acyl phospholipids. They were first isolated from the liver oil of Greenland shark but have also been found in other cartilaginous fish species, in sponge and mammals (Carballeira, 2002; Hallgren et al., 1974a,b; Hallgren and Stallberg, 1967; Hayashi and Takagi, 1982). They have been reported to exhibit antibiotic activity and inhibit dissemination and tumour growth in mice (Boeryd et al., 1971; Hallgren et al., 1978).

Archaea (archaeabacteria) possess a unique class of glycerol based ether lipids that are different from those found in most other species (Bacteria and Eucarya). The major ether lipid backbones found in archaeal cell membranes are 2,3-di-*O*-phytanyl-*sn*-glycerol diether, called archaeol **1**, and a glycerol dialkyl glycerol tetraether, called caldarchaeol **2** (Fig. 4). Surprisingly, the

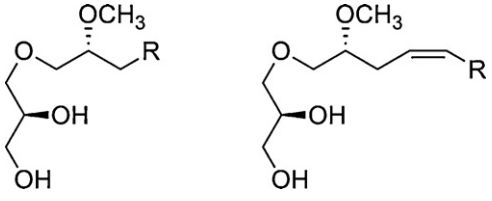


Fig. 3. Saturated and monounsaturated ether lipids of the (2′*R*)-1-*O*-(2′-methoxyalkyl)-*sn*-glycerol type.

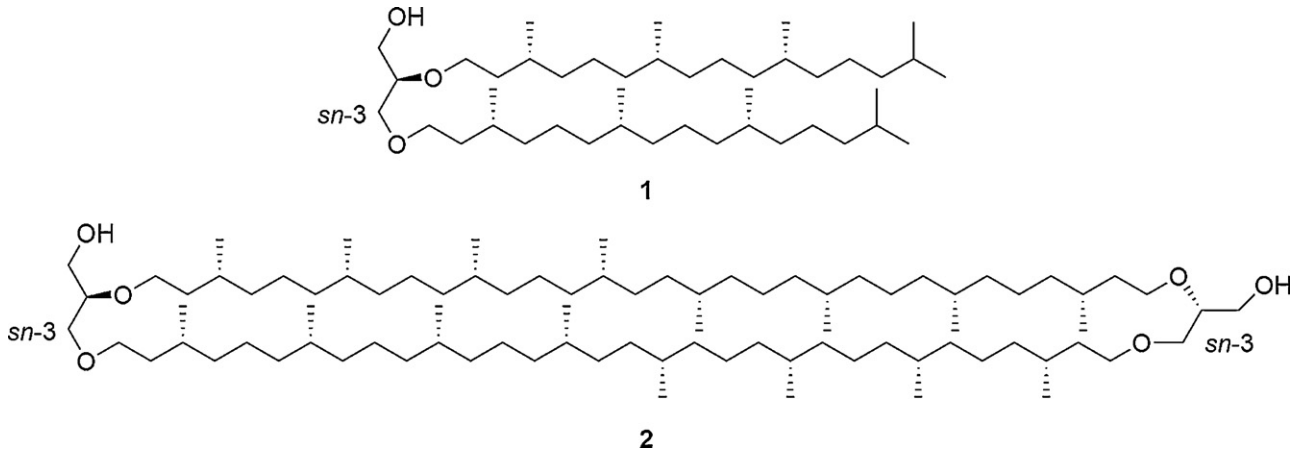


Fig. 4. Archaeol **1** and caldarchaeol **2**.

stereochemistry of their glycerol moiety is opposite to that found in Bacteria and Eucarya, that is, the end-positioned *O*-alkyl chain is located at the *sn*-3 position of the glycerol backbone instead of the *sn*-1 position (Aoki and Poulter, 1985; Heathcock et al., 1985; Koga et al., 1993). Archaeal ether lipid membranes have a broad heat and permeability range of stability (1–100 °C) and enable Archaea to survive in such harsh environmental conditions as extreme high temperatures, low pH and high metal ion concentrations which are their natural habitat (Koga and Morii, 2005). Archaeal ether lipids have found a variety of applications, including serving as antigen-carrying liposomes (due to their adjuvant properties) (Krishnan et al., 2000; Sprott et al., 2008), as boundary lubricants and in biomedical nano-coatings (Bode et al., 2008).

2. 1-*O*-alkyl-*sn*-glycerols

Kossel and Edlbacher (1915) were the first to isolate an alkylglycerol from the unsaponifiable fraction of the starfish *Asterias rubens* that much later was assigned as batyl alcohol **3** (Bergmann and Stansbury-Jr, 1943). Tsujimoto and Toyama (Heilbron and Owen, 1928), working with the unsaponifiable fractions from cartilaginous fish, isolated and recorded the chemical properties of the 1-*O*-alkyl-*sn*-glycerols comprising stearyl (C18:0), palmityl (C16:0), and oleyl (C18:1) alkyl chains and gave them the trivial names batyl **3**, chimyl **4**, and selachyl **5** alcohols in accordance with the chondrichthyans they were isolated from i.e. Batoidea (rays), Chimearas (ratfish), and Selachii (sharks), respectively (Fig. 5).

Further studies demonstrated that in marine oils 1-*O*-alkyl-*sn*-glycerols were present as fatty acid diesters, also called diacyl glyceryl ethers (Andre and Bloch, 1932), although, they were not isolated as such until 1960 (Mangold and Malins, 1960). The discovery of plasmalogens (Eichberg et al., 1961; Feulgen and Bersin, 1939) and their analogues plasmanyl-phospholipids (Carter et al., 1958) demonstrated that 1-*O*-alkyl-*sn*-glycerols were not only restricted to marine animal species, but they were in fact ubiquitous constituents of animal cell membranes. Furthermore, in the early 1970s Benveniste and Vargaftig (1983) characterized a platelet-activating factor (PAF), 1-*O*-alkyl-2-acetyl-*sn*-glyero-3-phosphocholine, being a group of highly versatile signal mediators found in very low concentration endogenously released by mammal cells involved in inflammatory reaction and reproductive functions (Roudebush et al., 2005).

The naturally occurring 1-*O*-alkyl-*sn*-glycerols, also referred to as alkylglycerols, are mainly found as 1-*O*-alkyl-2,3-diacyl-*sn*-glycerols and 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phospholipids. The next section focuses on the neutral diacyl glyceryl ethers and their 1-*O*-alkyl distribution in different animal species albeit paying special attention to the cartilaginous fish.

2.1. Occurrence

2.1.1. Ocurrence and distribution

In the early years after the discovery of 1-*O*-alkyl-*sn*-glycerols, the unsaponifiable matter obtained from alkaline hydrolysis of extracted fat was used for quantitative and qualitative analysis of alkylglycerols, due to lack of chromatographic methods not yet developed at that time; therefore, in many cases information about the original ether lipid classes they came from were missing. This can be seen in the isolation of alkylglycerols from the starfish *Asterias rubens* (Kossel and Edlbacher, 1915) and the coral related gorgonian *Plexaura flexuosa* (Kind and Bergmann, 1942).

Karnovsky and Rapson (1946) were the first to apply periodic acid to estimate the amount of alkylglycerols in unsaponifiable fractions based on the specific reaction of periodic acid with 1,2-diols assuming alkylglycerols were the only 1,2-diol, present in the unsaponifiable matter. By using this method Karnovsky et al. (1946) estimated the alkylglycerol content in oils from a wide range of animals of marine and terrestrial origin like teleost fish, molluscs, a crustacean, a reptile, an amphibian, a bird, some mammals, and from vegetable material. The study showed that alkylglycerols were widely spread among marine and land animals, although in

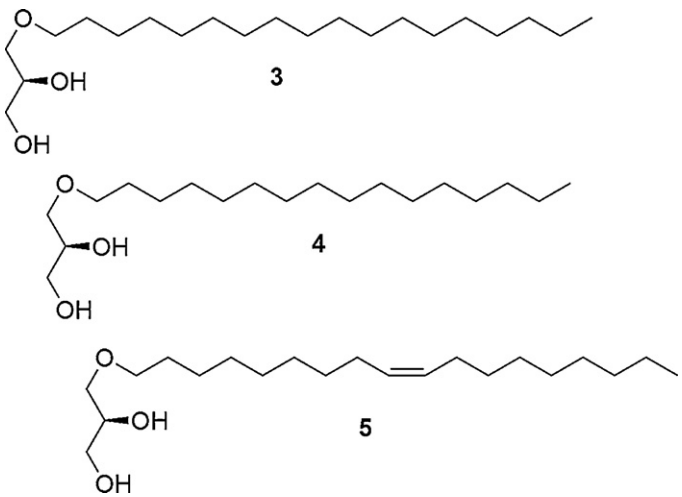


Fig. 5. The most prevalent 1-*O*-alkyl-*sn*-glycerols found in nature, batyl **3**, chimyl **4** and selachyl **5** alcohols.

low amounts, but were almost absent in plant cells. However, the presence of alkylglycerols has been proved in the pollen of *Pinus halepensis* (Andrikopoulos et al., 1985). Furthermore, Karnovsky et al. (1946) found appreciable amounts of alkylglycerols in the liver oil of the teleost fish *Austroglossus microlepsis*, the hepatopancreas and some viscera of the mollusc *Octopus rugosus* and in the flesh and stomach of the crustacean crayfish *Jasus lalandii*. They also found that the oils of invertebrate marine animals contained higher alkylglycerol levels than the oils from vertebrate land animals.

The introduction of chromatographic methods for the analysis of alkylglycerols made possible the separation of 1-*O*-alkyl-2,3-diacyl-*sn*-glycerols and alkyl acyl phosphatides from triacylglycerols (TAG) and diacylglycerophospholipids, respectively, facilitating enormously a more profound investigation on the 1-*O*-alkyl, fatty acid and polar head distribution. Among the molluscs the Antarctic isopods *Serolis pagenstecheri* and *Serolis cornuta* (Clarke, 1984) have been found to contain less than 1.5% DAGE and the tropical sea slug nudibranch *Phyllidia coelestis* (Zhukova, 2007) about 5% DAGE of their lipids. Surprisingly, DAGE have been found in particularly high amounts in the zooplankton sea slug *Clione limacina* (Kattner et al., 1998; Phleger et al., 1997) and in the liver oil of the gonatid squid *Berryteuthis magister* (Hayashi et al., 1985), 28–40 and 27.5% of their total lipids, respectively. The crustacean Antarctic krill *Euphausia superba* (Fricke et al., 1986) has been reported to contain 0.3–0.6% DAGE. Recently, the Boreal Soft Coral *Gersemia rubiformis* (Imbs et al., 2006) has been reported to contain up to 12% DAGE of the total lipids.

Hallgren et al. (1974a) reported the alkylglycerol content as a percentage of the neutral lipids and phospholipid fractions in the flesh of the teleost fish herring (*Clupea harengus*) and mackerel (*Scomber scomber*), in the crustaceans marine crayfish (*Nephrops norvegicus*), fresh-water crayfish (*Astacus fluviatilis*) and shrimps (*Pandalus borealis*), in the molluscs sea mussels (*Mytilus edulis*) and in cod liver oil. In this study the amount of alkylglycerols in the neutral lipids of the crustaceans and the molluscs were remarkably higher (10–20 times) than in the flesh of the teleost fish and cod liver oil. In addition, the study showed that 1-*O*-alkyl-*sn*-glycerols were more prevalent in the phospholipids than in the neutral lipids in all the samples investigated. For instance, alkylglycerols in herring flesh were about an order of magnitude higher in the phospholipid fraction (0.57%) than in the neutral lipid fraction (0.05%). Both marine crayfish and shrimps contained the highest amount of alkylglycerols in the neutral lipids, 1.2% (3.0% DAGE), and sea mussels contained the highest amount of alkylglycerols in the phospholipids, 1.9% (3.8% alkyl acyl phospholipids), among the organisms investigated.

Alkyl acyl phospholipids have been found in particularly high amounts in the phospholipids of the octopus *Octopus dofleini* (29.8%) and in the starfish *Asterias fortesi* (27%). Deeper look into the polar head distribution of the plasmanyl-phospholipids revealed that plasmanyl-ethanolamine accounted for 83% of the ethanolamine phospholipids (PE) in the sponge *Halichondria panicea*. Despite the scanty data concerning the amount of alkylglycerols in different phospholipid types it has been assumed that in aquatic invertebrates the 1-*O*-alkyl linked phospholipids are mainly found as ethanolamine and choline phospholipids, i.e. PE and PC (Chapelle, 1987).

In mammals, 1-*O*-alkyl-*sn*-glycerols are ubiquitously found in different tissues, although often as minor lipid components. Hallgren et al. (1974b) studied the occurrence of 1-*O*-alkyl-*sn*-glycerols in neutral and phospholipid fractions in cow, sheep and human milk, and in human bone marrow, red blood cells and blood plasma. They found that the percentages of alkylglycerols in phospholipids were usually much higher than in neutral lipids in all cases studied. Interestingly, human colostrums had a higher content of alkylglycerols than human milk, which was ten times higher than cow milk and twice as high as sheep milk. In this study the human bone marrow contained the highest percentage of alkylglycerols in both neutral, 0.33% (0.82% DAGE), and in phosphorylated fractions, 2.0% (4.0% alkyl acyl phospholipids) of the samples studied. Snyder and Wood (1969) found higher prevalence of alkylglycerols in phospholipid fractions compared to neutral lipid fractions in 16 of 19 different human tissues studied. They found that kidney and heart were richest in DAGE containing the highest percentages of alkylglycerols in neutral lipids, 1.7% and 2.5%, respectively, while brain was quite poor in DAGE and testes were completely lacking of them. Furthermore, colon and heart tissues showed the highest quantities of 1-*O*-alkyl linked phospholipids, 7.6% and 5.6%, respectively, while adipose tissues were quite poor in plasmanyl-phospholipids. This study also revealed the higher prevalence of 1-*O*-alkyl-*sn*-glycerols in the phospholipids compared to neutral lipid fractions in most human tissues. Similar trends have been reported from rat tissues (Rapport and Lerner, 1959).

Although Karnovsky et al. (1946) and Hallgren et al. (1974a,b) demonstrated low amounts of alkylglycerols in teleost fish compared to cartilaginous fish, Mori et al. (1972) found three species of teleost fish, *Cubiceps gracilis*, *Centrolophus* sp. and *Stromateus maculates* to possess DAGE as major lipid in their muscles. These fish contained high levels of oil in their flesh, 18.9, 21.0 and 15.2%, respectively, and remarkably high levels of DAGE about 60, 70 and 50%, respectively.

It is of interest to compare the occurrence of 1-*O*-alkyl-*sn*-glycerols in both neutral lipids and phospholipids with that of their 1-*O*-(alk-1′-enyl) analogues. In this regard, studies have shown that in human tissues 1-*O*-alkyl-*sn*-glycerol content is always higher than 1-*O*-(alk-1′-enyl)-*sn*-glycerol content in neutral lipids, whereas 1-*O*-(alk-1′-enyl)-*sn*-glycerols generally predominate in phospholipids (Albert and Anderson, 1977; Snyder and Wood, 1969). In addition, 1-*O*-alkyl-*sn*-glycerols are generally more prevalent in the choline phospholipids than in the ethanolamine phospholipids while 1-*O*-(alk-1′-enyl)-*sn*-glycerols are more prevalent in the ethanolamine phospholipids than in the choline phospholipids (Chabot et al., 1990; Mueller et al., 1984). For example, 1-*O*-alkyl linked phospholipids accounted for 42% of PC but only 10% of PE while 1-*O*-(alk-1′-enyl) linked phospholipids composed 70% of PE and only 5% of PC in human polymorphonuclear leukocytes (Chabot et al., 1990).

2.1.2. Liver oil of cartilaginous fish

The liver oil of various chondrichthyans (sharks, rays and chimaeras) are the major reservoir of naturally occurring 1-*O*-alkyl-*sn*-glycerols, and to date certain shark species caught by direct fishery or by-catch supply the demands from industry on shark liver oil due to their high content of squalene and alkylglycerols. The amount of alkylglycerols is variable, not only between species, but also among members of the same species. This variability might be related to different environmental factors such as depth and season, for example the DAGE content in the liver from *Lamna ditropis* was lower in summer compared to winter, 1.1–1.3% and 3.8–5.3%, respectively (Jayasinghe et al., 2003).

Studies have shown that certain orders of deep-sea sharks possess high liver to body weight ratios followed by high oil to liver ratios. For instance, some shark species of the Squaliformes (dogfish sharks) order possess liver to body weight ratios of about 20% and oil to liver weight ratios ranging from 65% for *Centroscymnus owstoni* (Owstonís dogfish) (Wetherbee and Nichols, 2000) to 86% for *Centrophorus squamosus* (Leafscale gulper shark) (Deprez et al., 1990). Hitherto, the highest DAGE amount reported in the liver oil of a chondrichthyan is from the Squaliforme *Scymnodon plunketi*, which contained 89% DAGE, 10% triacylglycerol and none squalene in its liver oil (Wetherbee and Nichols, 2000).

Table 1
DAGE and TAG % in the liver oil of *S. acanthias* from different places: (a) Kang et al. (1998), (b) Malins et al. (1965), (c) Wetherbee and Nichols (2000).

(%)	N. Atlantic[a]	N. Pacific[a]	N. Pacific[b]	New Zealand[c]
DAGE	18	41	45	12
TAG	81	59	47	87

The lipid compositions of some deep-sea Squaliformes from Australian, New Zealand and Tasmaninan waters are well documented and a compilation of the major lipids in their liver oils shows the mean percentages of DAGE ($24 \pm 7\%$), squalene ($65 \pm 11\%$) and TAG ($10 \pm 5\%$) for the following species: *Centroscymnus crepidater*, *Centroscymnus owstoni*, *Dalatias licha*, *Deania calcea*, *Etmopterus granulosus*, *Centrophorus crepidater*, *Centrophorus scalpratus*, *Centroscymnus coelolepis*, and *Etmopterus baxteri* (Bakes and Nichols, 1995; Deprez et al., 1990; Wetherbee and Nichols, 2000). High DAGE:TAG ratios are relevant for industry since in the process of DAGE extraction and refinement for health supplements DAGE and TAG are usually not separated. Therefore, some deep-sea Squaliformes with high DAGE:TAG ratios are potential sources of high quality shark liver oil for industry.

Other shark species that contain high amounts of DAGE and have been caught because of their liver oil include *Somniosus microcephalus* (Greenland shark) and *Squalus acanthias* (Spiny dogfish). *S. microcephalus* is a deep-sea shark that belongs to the Squaliformes order and has been reported to contain 30% DAGE in its liver (Hallgren and Larsson, 1962). *S. acanthias* inhabits both deep and shallow waters and has been reported to contain 12–45% DAGE in its liver, depending on their geographical habitat. *S. acanthias* is a good example of how DAGE can vary in a certain shark species, depending on its geographical habitat (Table 1). Furthermore, *Hexanchus griseus* (Bluntnose sixgill shark) (Wetherbee and Nichols, 2000) from the Hexanchiformes order, which is a relatively deep-sea shark, and *Galeocerdo Cuvier* (Tiger shark) (Navarro-Garcia et al., 2000), a shallow-water species which belongs to the Carcharhiniformes order, have been reported to contain high levels of DAGE in their liver oils, 70% and 30%, respectively.

Cartilaginous fish species of the Holocephali subclass and under the Chimaeridae order, to which all the living ratfish species belong, contain high amounts of DAGE. For instance, the ratfish species *Hydrolagus novaezealandiae*, *Hydrolagus barbouri* and *Rhinochimaera pacifica*, have been shown to posses high amounts of DAGE (60%) in their liver oils, which is higher than found in DAGE rich deep-sea sharks (Hayashi and Takagi, 1982). In addition, *Chimaera monstrosa* has been reported to contain almost exclusively DAGE in its liver (Hallgren and Larsson, 1962). Some deep-sea shark species of the Apristurus genus have been observed to accumulate low to moderate amounts of DAGE (<1–13%) in their livers (Wetherbee and Nichols, 2000). Shallow-water shark species are generally known to possess small livers with lower lipid yields than deep-sea sharks (Sargent et al., 1973; Van Vleet et al., 1984). The pelagic shark *Cetorhinus maximus* (basking shark) has a big liver, mainly containing TAG (68%) and squalene (27%), but almost deprived of DAGE (Lombardi et al., 1971). Some shallow-water sharks and rays contain DAGE, although in low amounts. For instance, *Lamna ditropis* (Jayasinghe et al., 2003), *Carcharinus falciformis* (Jaqueton) (Navarro-Garcia et al., 2000), and the ray *Dasyatis brevis* (Navarro-Garcia et al., 2004) contained about 3, 7 and 9% DAGE in their liver oils, respectively.

2.1.3. Occurrence in cancer cells

Snyder and Wood (1969) studied the occurrence of 1-*O*-alkyl and 1-*O*-(alk-1′-enyl) linked ether lipids in 17 different human tumours and found the total ether content in the neutral lipid fraction to be higher in neoplastic cells than in most healthy cells, with the exception of heart and kidney. The levels of plasmanylphospholipids and plasmalogens were also generally higher in tumour cells than in many normal tissues. The same trends have been reported in animal experiments, where higher levels of ether lipids, and especially neutral ether lipids, were found in transplantable mouse and rat tumours, than in non-cancerous tissue (Snyder and Wood, 1968).

Lin et al. (1978) found DAGE levels to be on average higher in human hepatocellular carcinoma than in tumour-free residual tissue or in noncancerous liver. Indeed, a deeper look into the 1-*O*-alkyl composition revealed that chimyl alcohol was the most abundant alkylglycerol in carcinoma, and that strikingly different ratios were present between the most prevalent alkylglycerol types chimyl, batyl and selachyl alcohols, 2:1:1 in the neoplastic cell, but 1:2:2 in the normal cells. Therefore, the proportion of saturated to monounsaturated alkylglycerols was increased by more than twice.

Merchant et al. (1991) have reported higher concentrations of choline plasmalogen in malignant human breast tissues compared to healthy breast tissue. Albert and Anderson (1977) have also reported high levels of choline plasmalogens, plasmanylphospholipids, neutral plasmalogens and DAGE in various human brain tumours compared to normal brain tissues. On the other hand, Chabot et al. (1990) reported lower levels of plasmanylphosphocholine in the choline phospholipid fraction from patients suffering from acute myelogenous leukemia (10–20%), compared to normal polymorphonuclear leukocytes (42%). Furthermore, the levels of plasmanylcholine in leukemic cells were positively correlated to their degree of cellular differentiation. In the same study the levels of ethanolamine plasmalogen in human leukemia cells (42–55%) were also reduced, compared to normal leukocytes (66%) but to a lesser extent.

Gray (1963) analysed the total lipid composition of two tumour cells, Landschutz ascites-carcinoma cells and BP8/C3H ascites-sarcoma cells, with especial reference to the fatty acids and phospholipids. He found that the phospholipid composition, including the plasmalogens of the tumour cells, did not show marked differences from the composition of non-tumour cells. However, he noticed increased similarity in the 1-*O*-(alk-1′-enyl) composition of plasmalogens and in their fatty acid composition between the tumour cells investigated, suggesting a loss of selectivity of fatty acyl-CoA reductases and of acyl tranferases involved in plasmalogen formation. This is in agreement with the idea that tumour cells, unlike normal cells, tend to resemble each other chemically.

As can be seen, many studies have presented relatively high glycerol based ether lipid levels in mammal tumour cells compared to normal cells, therefore, indicating that increases in ether lipids may be characteristic of human neoplastic cells. However, some studies have reported no changes in the ether lipid composition, or even lower ether lipid concentrations in cancerous cells, than in normal cells. Therefore, it has not been possible to apply the relative increased ether lipid levels in medical diagnosis of cancer; perhaps a difficult achievement, since cancer may be regarded as many different and unrelated diseases. Nevertheless, it may be concluded that tumour cells are characterized by changes in their ether lipid fractions. These changes often implicate increase in ether lipid levels, probable variations in the 1-*O*-alkyl and 1-*O*-(alk-1′-enyl) compositions, and may alter the composition of the fatty acids located at the *sn*-2 position of the glycerol moiety. The reasons for these changes in the ether lipid fractions in tumour cells are still not known. These changes may be a consequence of impairing in the enzymes involved in ether lipid biosynthesis which may implicate changes in cell membrane fluidity influencing the function of membrane proteins altering cell signalling in tumour cells.

2.1.4. 1-O-alkyl-sn-glycerols and buoyancy control in marine animals

Despite of being essential constituents of cell membranes and serving as energy storage, high contents of alkylglycerols present in the livers of some deep-sea sharks are believed to play a significant role in maintaining neutral buoyancy during transversal migration. Various deep-sea shark species of the Squaliformes order accrue high amounts of low-density lipids such as squalene (density 0.86 g/ml) and DAGE (density 0.89 g/ml) in their liver. Squalene and DAGE provide 80% and 14% more uplift per unit volume in seawater than TAG (density 0.92 g/ml), which is the most abundant form of lipid storage in animals. Wetherbee and Nichols (2000) reported high amounts of squalene and DAGE in the liver of deep-sea Squaliformes caught at Chatham Ries in New Zealand and found that the sum of both compounds made up 90% of the liver lipids. Furthermore, they found that the amounts of squalene and DAGE in liver oil were inversely related.

Malins and Barone (1970) realized that DAGE exhibited an active turnover in the liver oil of the shark *Squalus acanthias*, and suggested that DAGE metabolism was related to the role of the liver as a hydrostatic organ. They artificially increased the body weight of a number of *S. acanthias* sharks for 50 h, and then compared their TAG and DAGE content in the liver to those of unweighted members. The results revealed that DAGE were significantly increased in weighted sharks and that TAG predominated in the unweighted sharks. Therefore, they have postulated that the weighted sharks increased their DAGE amounts in their livers to compensate the increase in body weight. In this manner, *S. acanthias* lack of swim bladder may be counterbalanced by a system based on regulation of DAGE to TAG ratios in the liver to obtain neutral buoyancy. Phleger et al. (1997) have indeed proposed a buoyancy function of DAGE in the Southern Ocean pteropod *Clione limacina*, which contains high amounts of DAGE (28%) in their lipids compared to the pteropod *Limacina helicina*, which lacks DAGE.

2.2. Biosynthesis and 1-O-alkyl chain composition

All the biosynthetic steps involved in the formation of glycerol ether lipids have been established and studied profoundly, mainly in mammal cells. The biosynthesis of 1-*O*-alkyl and 1-*O*-(alk-1′-enyl) glycerol based ether lipids (Scheme 1) begins with the acylation of dihydroxyacetone phosphate (DHAP) with a long-chain acyl-CoA ester, catalyzed by dihydroxyacetone phosphate acyltransferase (DHAP-AT), an intraperoxisomal enzyme.

In the second step the characteristic ether bond of glycerol ether lipids is formed by replacement of the *O*-acyl group by the *O*-alkyl group of a long chain fatty alcohol at the *sn*-1 position of 1-acyl-dihydroxyacetone phosphate (1-acyl-DHAP), yielding the first ether linked intermediate, i.e. 1-*O*-alkyl-dihydroxyacetone phosphate (1-*O*-alkyl-DHAP). This remarkable reaction catalyzed by the membrane bound peroxisomal enzyme alkyldihydroxyacetone synthase (alkyl-DHAP-S) has been studied extensively, albeit its mechanism is still not known (Brown and Snyder, 1982).

One of the most peculiar features of this reaction is that the oxygen in the ether linkage is derived from the fatty alcohol, implying that the replacement of the acyl-group occurs through the breakdown of the chemical bond between the glycerol carbon atom at the *sn*-1 position of 1-acyl-DHAP and the *O*-acyl group of the fatty ester. Recently, it has been reported that alkyl-DHAP-S contains a flavin adenine dinucleotide (FAD) molecule as cofactor, that is essential for the enzyme activity (de Vet et al., 2000). The fatty alcohols are obtained by reduction of long-chain acyl-CoAs to alcohols by an acyl-CoA reductase.

Subsequent reduction of the resulting 1-*O*-alkyl-DHAP leads to the first glycerol based intermediate 1-*O*-alkyl-*sn*-glycero-3-phosphate (1-*O*-alkyl-G3P) carried out by acyl/alkyldihydroxyacetone phosphate reductase (acyl/alkyl-DHAP-R), aided by the cofactor NADPH.

Further esterification of the resulting intermediate with acyl-CoA at the *sn*-2 position yields 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphate (1-*O*-alkyl-2-acyl-G3P) catalyzed by an alkyl-acyl-glycero-3-phosphate acyltransferase (alkyl-acyl-G3P-AT). After the removal of the phosphate group from the *sn*-3 position of 1-*O*-alkyl-2-acyl-G3P by a phosphohydrolase (PH), the fate of the resulting 1-*O*-alkyl-2-acyl-*sn*-glycerol may follow two different metabolic pathways, depending on whether the final product is a neutral DAGE or an ether phospholipid. 1-*O*-Alkyl-2-acyl-*sn*-glycerol can be esterified with a long-chain acyl-CoA ester by an acyltransferase (AT), yielding 1-*O*-alkyl-2,3-diacyl-*sn*-glycerol (DAGE), or it can be converted to 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (plasmanyl-ethanolamine) by reaction with cytidine-diphosphate-ethanolamine (CDP-ethanolamine) via the action of ethanolamine-phosphotransferase (ethanolamine-PT) in the presence of magnesium ions (Brites et al., 2004).

The plasmanyl-ethanolamine can further be transformed to their corresponding plasmenyl-ethanolamine through the action of Δ1-alkyl desaturase, that requires a cytochrome b_5 reductase-dependent electron transport system. Hitherto, the biosynthesis of plasmenyl-choline is not well understood, although radiolabeling studies have strongly suggested that plasmenyl-cholines arise from their plasmenyl-ethanolamine congeners. In this regard, the action of phospholipase A2, which is highly specific for plasmalogens, may be important for the elucidation of the biosynthetic pathway (Brites et al., 2004; Snyder, 1999). 1-*O*-(Alk-1′-enyl)-2,3-diacyl-*sn*-glycerol (neutral plasmalogens) are believed to be generated from their polar analogue plasmenyl-ethanolamine by removal of the polar head, achieved either by phospholipase or by the reverse reaction of ethanolamine-PT. The resulting 1-*O*-(alk-1′-enyl)-2-acyl-*sn*-glycerol is then esterified with acyl-CoA, yielding the diacyl derivative (Paltauf, 1983a).

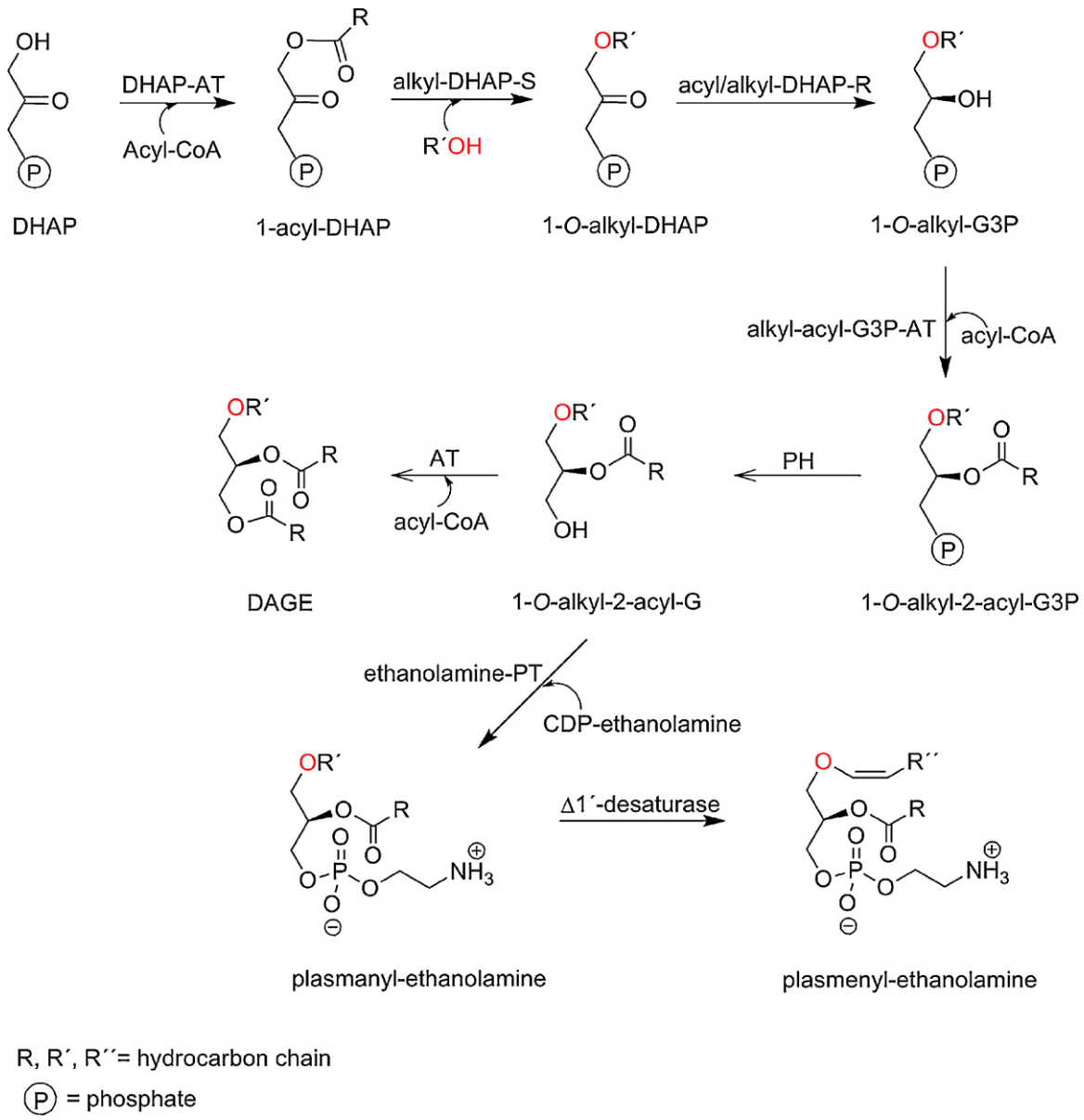
The 1-*O*-alkyl chain composition of DAGE has primarily been studied in the liver oil of chondrichthyans, in the DAGE rich flesh of teleost fish, marine invertebrates and in tumour cells. Table 2 reveals the 1-*O*-alkyl distribution in DAGE from 15 different species taken from different studies. The species included are five sharks, four teleost fish, two molluscs, two crustaceans, cow milk, human milk and human bone marrow. Inspection of Table 2 reveals that in general the alkyl chains contain even number of carbons ranging from C14 to C22, and they are almost entirely saturated or monounsaturated. Saturated and monounsaturated *O*-alkyl chains of 16 and 18 carbon atoms account for about 80% of the alkylglycerols found in DAGE. Odd-numbered, polyunsaturated and branched chains are only minor components. Alkyl chains shorter than 14 carbons are often found in teleost fish, although in less than 2%. Alkyl chains of 24 carbons have been found in samples from human origin, including milk and bone marrow. The dienoic alkyl chain C18:2 is the most prevalent polyunsaturated alkyl chain encountered among the unsubstituted alkylglycerols of the *sn*-1 configuration. This dienoic alkylglycerol seems to be restricted to organisms of marine origin, although mainly to chondrichthyans. A very rare diunsaturated C20:2 alkyl chain has been reported in the DAGE of marine crayfish. Branched alkyl chains seem to be prevalent in the milk of ruminants (Hallgren et al., 1974b) such as cows, where they account for about 10% of the alkylglycerols. The sea slug *Clione limacina* (Phleger et al., 1997) contains high levels of odd-number alkylglycerols (32%) including 21% of 1-*O*-pentadecyl glycerol, which is normally found in low amounts such as in the liver oil of the deep-sea Squaliformes *Centroscyllium ritteri* and *Somniosus microcephalus*, where it accounts for less than 1% of the alkylglycerols.

Selachyl alcohol (C18:1n-9) is by far the most abundant alkylglycerol present in the liver oil from deep-sea sharks of the Squaliformes order. In this regard, Deprez et al. (1990), Bakes and

Table 2
1-*O*-alkyl chain composition of DAGE from various animal species: (a) Kayama et al. (1971), (b) Malins et al. (1965), (c) Hallgren and Larsson (1962), (d) Mori et al. (1972), (e) Hallgren et al. (1974a), (f) Hayashi et al. (1985), (g) Phleger et al. (1997), (h) Hallgren et al. (1974b).

Alkyl chain	*C. ritteri* liver[a]	*S. acanthias* liver[b]	*S. acanthias* flesh[b]	*S. micro-cephalus* liver[c]	*C. mon-strosa* liver[c]	*C. maximus* liver[a]	*C. gracilis* flesh[d]	*S. maculatus* flesh[d]	Baltic herring flesh[e]	Squid liver[f]	Sea slug[g]	Marine crayfish[e]	Shrimps[e]	Cod liver oil[e]	Cow milk[h]	Human milk[h]	Human bone marrow[h]
9:0								0.1									
10:0							0.3	0.1									
10:1						0.4											
11:0							0.5	0.3									
12:0							0.5	0.1	0.25					1.4			
12:1						0.2	0.2		0.25								
13:0							0.3										
14:0 br	0.1					0.1									1.8		
14:0	2.8	2.6	4.8	2.0	1.7	6.5	4.3	9.1	13.9	1.1		3.8	4.8	5.5	5.8	0.9	0.5
14:1	0.8					3.2			6.4			0.6	0.5	15.5			
15:0 br	0.5	0.6	0.9												4.5		
15:0	0.9	0.7	1.0	0.7	1.1	3.1	2.1	1.4	1.2	0.7	21	3.2	2.8	0.5	3.2	0.5	0.4
15:1												1.6		1.9			
16:0 br	0.2	0.7	0.8							0.2					4.2		
16:0	15.3	13.4	22.9	9.1	10.4	35.9	40.3	68.7	57.7	58.7	60	36.4	44.5	13.4	27.8	24.8	33.2
16:1	6.5	10.2	13.2	10.8	9.1	11.6	5	2.8	3.9	0.5	3	12.6	8.4	19.2	1.4	3.2	
17:0 br		0.9	0.5							1.6	5						
17:0	2.0	0.3	0.8	3.6	4.7	1.7	2.8	1.6	0.1	1.7	5	2.7	2.9	1.8	0.9	1.4	1.1
17:1		1.1	0.8				0.7					2.2	3.7	2.2	1.8	1.6	
18:0 br	0.7	0.7	0.3							1.0	<1						
18:0	4	3.9	2.8	2.8	6.7	4.7	7.7	2.7	1.6	6.6	1	10.8	10.9	2.7	29.5	21.8	27.0
18:1n-9	54.2	61.4	47.3	59.4	53.6	30.6	33.7	13.1	8.8	24.6	1	12.4	10.3	23.2	17.7	37.5	20.3
18:2	1.8		0.7	1.6	2.4	0.2	0.2					2.4					
19:0 br											1						
19:0	3.6	0.8	0.3	1.5	2.4	0.5				0.9		0.5	0.2	0.1	0.1	0.1	0.2
19:1									0.1	0.8		1.2	0.9			0.4	0.2
20:0	1.6				0	0.2			0.4			0.6	0.4	0.2	0.2	0.9	3.0
20:1	4.8	0.2	0.1	6.2	6.4	0.7	1.4		4.1	1.5	3	5.9	12.3	8.2	0.3	1.7	4.2
20:2												0.7					
21:0 and 21:1									0.2							0.3	0.2
21:0	0.4													0.1			
21:1													0.3	0.1			
22:0														0.7		0.7	1.4
22:1		2.3	1.9	2.2	1.0	0.1			1.1			2.4		2.6		2.7	5.5
23:0 and 23:1																0.1	
24:0																	0.2
24:1																1.4	2.6
Saturated, straight	31	22	33	20	27	53	59	84	75	70	87	58	67	26	68	51	67
Monoenoic	66	75	63	79	70	47	41	16	25	27	7	39	36	73	21	49	33
Polyenoic	1.8	0.0	0.7	1.6	2.4	0.2	0.2	0.0	0.0	0.0	0.0	2.4	0.0	0.0	0.0	0.0	0.0

br: iso- and anteiso chains.



Scheme 1. The biosynthesis of 1-*O*-alkyl and 1-*O*-(alk-1′-enyl) glycerol based ether lipids.

Nichols (1995), Kayama et al. (1971), and Bordier et al. (1996) have reported the following percentage of selachyl alcohol: 40, 42–54, 76 and 42–62%, respectively. Indeed, Table 2 indicates that selachyl alcohol is also the major alkylglycerol in DAGE from the liver oil of the spiny dogfish *Squalus acanthias* and the rat fish *Chimaera monstrosa*. In all these cartilaginous fish the levels of chimyl alcohol (C16:0) and C16:1n-7 alkylglycerol made up about 20% of the alkylglycerols and the remaining was made up of batyl alcohol, C20:1 and C22:1 alkylglycerols. It is noteworthy, to point out that high levels of C20:1 alkylglycerol have been reported in *Squalus acanthias* and a *Centrophorus* species, 18 and 13%, respectively, showing fluctuations in the levels of certain alkylglycerol types in the liver oil of elasmobranch fish (Kang et al., 1998; Kayama et al., 1971).

The 1-*O*-alkyl chain composition of the liver oil of the basking shark (*Cetorhinus maximus*) is similar to that of other chondrichthyans mentioned before although there, the most prevalent alkylglycerols are chimyl and selachyl alcohols found at similar levels. Malins et al. compared the 1-*O*-alkyl chain composition in DAGE from the liver oil and flesh of the *Squalus acanthias* (Malins et al., 1965) (Table 2). The study revealed that the flesh contained higher quantities of chimyl alcohol compared to the liver; furthermore, the concentration of selachyl alcohol was lower in the flesh than in the liver.

Different from most cartilaginous fish species, some teleost fatty fish have chimyl alcohol as their main alkylglycerol component in their flesh fat, and the same can be said about the mollusc gonatid squid *Berryteuthis magister* and the zooplankton *Clione limacina*. In these species chimyl alcohol is by far the most abundant alkylglycerol, and its levels seem to increase on the expense of selachyl alcohol and of C16:1n-7 alkylglycerol, therefore, increasing the total amount of saturated alkylglycerols up to about 75%, which is high compared to about 25% generally found in the liver oil of cartilaginous fish (Table 2). Chimyl alcohol is also the most prevalent alkylglycerol found in crustaceans like marine crayfish (*Nephrops norvegicus*) and shrimp (*Pandalus borealis*) (Hallgren et al., 1974a). In mammals the 1-*O*-alkyl composition of DAGE is characterized by similar percentages of batyl, chimyl and selachyl alcohols, which together account for approximately 80% of the alkylglycerols. This implies a total amount of saturated alkylglycerols of about 60%, which is higher than found in cartilaginous fish, but less than found

in bony fish (Table 2). The saturated chimyl and batyl alcohols have been identified as the major alkylglycerols found in the marine sponge *Desmapsamma anchorata* (Quijano et al., 1994).

Although we have only discussed the alkyl composition in DAGE, studies have shown that the 1-*O*-alkyl composition in DAGE and in ether phospholipids is usually similar, but some differences are noticed in the percentages of the most abundant alkylglycerols (Gray, 1963; Hallgren et al., 1974a,b).

The narrow range of 1-*O*-alkyl chains found, together with the fact that 80% of them is composed of saturated and monounsaturated C16 and C18 alkyl chains, shows that alkylglycerols are synthesized by a common biosynthetic pathway which selectively chooses the fatty alcohol precursors based on their chain length and unsaturation. Studies on substrate specificity of the enzymes catalyzing the biosynthesis of ether lipids reveal that the enzyme alkyl-DHAP-S, which actually catalyzes formation of the ether bond from 1-acyl-DHAP and a fatty alcohol, utilizes a wide range of alcohols and does not appear to possess the specificity to account for the narrow spectrum of chains normally found in 1-*O*-alkyl-*sn*-glycerols of ether lipids (Wykle et al., 1979). On the other hand, some studies indicate that fatty acyl-CoA reductases, whose role is the synthesis of fatty alcohols from the corresponding fatty acids, reduces more easily the fatty acids palmitic, stearic and oleic acids than saturated shorter or longer polyunsaturated fatty acids (Reichwald-Hacker, 1983).

Recently, two mammalian acyl-CoA reductase isoenzymes, FAR1 and FAR2, have been identified and expressed in intact cells. The fatty acid preferences of the enzymes were investigated and FAR1 showed a relatively broad specificity with regards to fatty chain length and unsaturation. For instance, that enzyme converted the fatty acids comprising C16:0, C18:0, C18:1 and C18:2 chains to their corresponding alcohols, but left out the shorter and longer polyunsaturated fatty acids. Indeed, FAR2 showed a strong specificity toward the saturated fatty acids of 16 and 18 carbon chains. Another interesting fact is that these two isoenzymes have different tissue distribution (Cheng and Russell, 2004; Hartvigsen et al., 2006). Therefore, the 1-*O*-alkyl composition appears to be determined primarily by fatty acyl-CoA reductases of different fatty acid selectivity. Both isoenzymes prefer fatty acyl-CoA of 16 and 18 carbon chains; one of them (FAR2) is specific toward palmityl- and stearyl-CoA substrates and the other (FAR1) accepts also the monounsaturated analogues palmitoleyl- and oleyl-CoA and the polyunsaturated linoleyl-CoA. These discoveries explain the occurrence of C16 and C18 saturated and monounsaturated 1-*O*-alkyl chains as the main components of alkylglycerols in animal cells, and the low amounts of shorter or longer alkyl chains. Furthermore, different tissue distributions of reductases may explain varying concentrations of the major alkylglycerols in different animal tissues.

In the liver oil of *Squalus acanthias* the C16:1 and C18:1 fatty acid percentages in DAGE, 3.8% and 21.2%, respectively, do not reflect the occurrence of the C16:1 and C18:1 long alkyl chains of the alkylglycerols, 10.6% and 47.8%, respectively, inferring the specificity of fatty acyl-CoA reductases in sharks. Furthermore, relatively high percentages of C20:1 (13.4%) and C22:1 (23.6%) fatty acids characteristic of DAGE are only found in low amounts, 8% and 2.7%, respectively, in the corresponding 1-*O*-alkyl chains of the alkylglycerols. Low percentages of alkyl chains longer than 18 carbon atoms might indicate decreasing substrate tolerance of fatty acyl-CoA reductases toward fatty acids longer than 18 carbon atoms. Similar trends have been reported in the liver oil from *Somniosus microcephalus*, *Chimaera monstrosa* and deep-sea sharks (Hallgren and Larsson, 1962; Hartvigsen et al., 2006). In the teleost fish *Siorella* sp. the proportion of the C20:1 alkyl chain of the alkylglycerols (25%) is higher than that of the corresponding fatty acid (14%) in the diacyl derivative. In the liver oil of *Berryteuthis magister* and in the sea slugs *Clione limacina* and *Spongiobranchaea australis* the percentages of chimyl alcohol are seven, five and three times higher, respectively, than that of the corresponding palmitic acid in the DAGE. Furthermore, in *Clione limacina* the C15:0 alkylglycerol content is 21% while the pentadecanoic acid accounts only for less than 2% of the fatty acid composition of the diesters, therefore indicating that some reductases might be showing a fatty acid preference for pentadecanoic acid (Hayashi et al., 1985, 1978; Phleger et al., 1997). These results show that the 1-*O*-alkyl-*sn*-glycerol composition is strongly monitored by certain fatty acyl-CoA reductases, and that the 1-*O*-alkyl composition of alkylglycerols does not reflect the fatty acid composition of DAGE, which is similar to that of TAG.

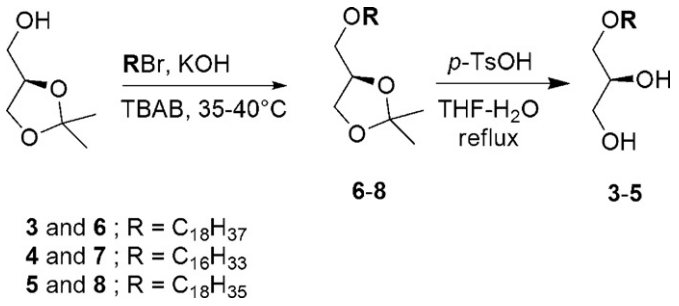
2.3. Chemical synthesis

The first syntheses of 1-*O*-alkylglycerols were accomplished to elucidate their chemical structure and later on to determine their stereochemistry. The first synthesis of 1-*O*-alkylglycerols was performed by Davies et al., who synthesized racemic chimyl and batyl alcohols by way of allyl ethers that were readily obtained by heating the required alkyl chloride with sodium allyloxide in allyl alcohol solution (Davies et al., 1930). The alkylglycerols were obtained after oxidation of the double bond with hydrogen peroxide in glacial acetic acid.

Fischer and co-workers (Baer and Fischer, 1941; Baer et al., 1944) were the first to prepare batyl, chimyl and selachyl alcohols, and their antipodes, enantiomerically pure by using the chiral C_3-synthons, (*S*)-1,2-*O*-isopropylidene-*sn*-glycerol obtained from D-mannitol, and (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol prepared from L-arabinose (Baer and Fischer, 1939a). Knowing the predetermined stereochemistry of the synthetic compounds and their optical rotations, they were capable to determine the *S*-configuration of the naturally occurring 1-*O*-alkyl-*sn*-glycerols. They synthesized (*S*)-chimyl and (*S*)-batyl alcohols and their corresponding enantiomers by refluxing the sodium salts of (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol and (*S*)-1,2-*O*-ispropylidene-*sn*-glycerol, obtained via sodium naphthalene, with hexadecyl and octadecyl iodide in glycol dimethyl ether.

Subsequent hydrolysis of the acetonides, by heating at 80 °C in 80% acetic acid for 2 h, afforded the desired products in about 65 and 54% overall yields for the octadecyl and hexadecyl derivatives, respectively. Fischer's group (Baer et al., 1944) also accomplished the synthesis of selachyl alcohol and its antipode, using the same methodology as for the synthesis of batyl and chimyl alcohols. This time, however, they used *p*-toluenesulfonate of oleyl alcohol instead of halide, assuming the *p*-toluenesulfonates to be more reactive and more readily prepared, without affecting the double bond, than the halides. They reported about 80 and 76% overall yields for the *S* and *R* enantiomers of selachyl alcohol, respectively.

Baumann and Mangold (1964) performed the synthesis of 1-*O*-alkylglycerols via condensation of 1,2-*O*-isopropylideneglycerol with alkyl methanesulfonates in the presence of potassium in boiling benzene or ground potassium hydroxide in boiling xylene. The ketal moiety was subsequently removed by acid-catalyzed hydrolysis in 10% HCl in methanol. The employment of potassium metal and potassium hydroxide gave high yields for the synthesis of 1-*O*-alkylglycerols with long saturated alkyl chains, about 77% overall. Likewise, the yields for the unsaturated 1-*O*-alkylglycerols were slightly lower, about 63 and 49% for potassium and potassium hydroxide, respectively. This method has some advantages compared to Fischer's method, for instance, the preparation of alkyl methansulfonates being easier than alkyl *p*-toluenesulfonates, among other things, because the methansulfonate esters were more readily crystallized according to the authors. Furthermore, in Baumann and Mangold's method the alkylglycerols are more easily isolated from the crude mixture, than by



Scheme 2. Synthesis of (*S*)-batyl **3**, (*S*)-chimyl **4** and (*S*)-selachyl **5** alcohol.

following Fischer's method, where naphthalene and dihydronaphthalene side-products made the isolation of alkylglycerols more tedious.

More recently, Urata et al. (1988) have achieved the synthesis of 1-*O*-alkylglycerols via prior preparation of alkyl glycidyl ethers (1-*O*-alkyl-2,3-epoxypropanes), by coupling epichlorohydrin with fatty alcohols using a quaternary ammonium salt as phase transfer catalyst in an alkaline water-hexane solvent system, resulting in 80% yield for the oleyl chain. The epoxide was then converted to the corresponding acetonide or diacetate by treatment with acetone and boron trifluoride etherate ($BF_3{\cdot}Et_2O$) or acetic acid anhydride with a tertiary amine, followed by hydrolysis, in 63 and 67% overall yields, respectively. This method has the great disadvantage of being limited to the synthesis of racemic 1-*O*-alkylglycerols. The non-selective attack of the nucleophile, both to the less substituted carbon of the epoxide ring and the methylene carbon bearing the halide, will result in racemisation of the alkyl glycidyl ethers in case enantiopure epichlorohydrin to be used.

An efficient approach has been developed to prepare batyl, chimyl and selachyl alcohols enantiomerically pure (Magnusson et al., 2011) and as racemates (Halldorsson et al., 2004). The synthetic approach is shown in Scheme 2 for the synthesis of the naturally occurring (*S*)-batyl **3**, (*S*)-chimyl **4** and (*S*)-selachyl **5** alcohols. (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol was reacted with the corresponding stearyl, palmityl and oleyl bromide, in stoichiometric amounts, in the presence of ground potassium hydroxide and catalytic amount of tetra-*n*-butylammonium bromide (TBAB) under solvent free conditions at 35–40 °C for 24 h. The acetonide intermediates **6–8** were not isolated, but subsequently deprotected by mild-acidic hydrolysis of the isopropylidene moiety in a tetrahydrofuran-water solution in the presence of *p*-toluenesulfonic acid. The 1-*O*-alkyl-*sn*-glycerols **3–5** were finally afforded after crystallization from *n*-hexane or by chromatography in 77–80% yields from (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol.

The enantiopure chimyl, batyl and selachyl alcohols **3–5** were introduced to a highly efficient two-step chemoenzymatic synthesis of enantiopure structured ether lipids of the DAGE type possessing a pure saturated fatty acid (SFA) at the *sn*-3 position and pure EPA and DHA attached to the *sn*-2 position. Full regiocontrol was offered by use of an immobilized *Candida antarctica* lipase that operated highly efficiently at room temperature. This has been extended to preparation of a focused library of such structured ether lipids (total of 72 compounds) possessing all even-numbered SFA ranging from C_2–C_{16} (Magnusson et al., 2011).

2.4. Isolation, identification and structural elucidation

The isolation of DAGE usually begins with total extraction of lipids from tissue samples by the methods developed by Bligh and Dyer (1959) or Folch et al. (1957). Then, the DAGE fraction is separated from the total lipid extract by preparative thin layer chromatography (TLC) or column chromatography on silicic acid or silica gel (Hartvigsen et al., 2006; Kang et al., 1998; Malins et al., 1965; Sargent et al., 1973). After alkaline hydrolysis of the DAGE fraction the obtained 1-*O*-alkyl-*sn*-glycerols are derivatized to their corresponding isopropylidene (Kang et al., 1998; Malins et al., 1965), diacetylated (Sargent et al., 1973), disilylated (TMS) (Kang et al., 1998) or dimethoxylated (Hallgren and Larsson, 1962) derivatives prior to gas–liquid chromatography (GLC), equipped with flame ionization detector (FID) (Hallgren et al., 1974b; Hartvigsen et al., 2006; Kang et al., 1998), for analysis of the 1-*O*-alkyl chain profile. For further confirmation of the 1-*O*-alkyl-*sn*-glycerol peaks, GLC-mass spectrometry (GLC-MS) with an ion trap detector (ITD) has been applied (Kang et al., 1998).

More recently, an HPLC method utilizing evaporative scattering detection (ELSD) has been described for the separation and analysis of 1-*O*-alkylglycerols and their acylated derivatives in non-polar lipid fractions (Torres et al., 2005). Furthermore, a reversed-phase HPLC coupled to electrospray ionization/collision-induced dissociation/mass spectrometry (ESI/CID/MS) has been used to separate and analyse different DAGE species (Hartvigsen et al., 2001, 2006).

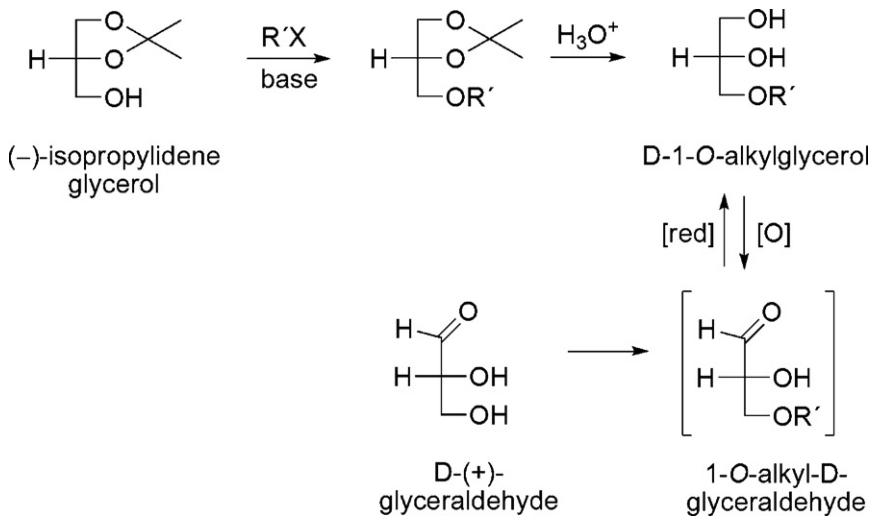
As pointed out in the previous section, Fischer's group was the first in the 1940s to synthesize both antipodes of batyl, chimyl and selachyl alcohols enantiomerically pure from the corresponding isopropylideneglycerol enantiomers. The enantiopure precursors were obtained from appropriate carbohydrates of the chiral pool. Their work demonstrated that the optical rotation values of the 1-*O*-alkylglycerols, obtained from the liver oil of some cartilaginous fish species, and their acetonide and diacetate derivatives, agreed with those of the 1-*O*-alkylglycerols synthesized from (−)-isopropylideneglycerol (Baer and Fischer, 1941; Baer et al., 1944).

Since the absolute configuration of any chiral compound was at that time unknown, including that of (−)-isopropylideneglycerol, the sense of chirality of the 1-*O*-alkylglycerols was indeed obscure. Instead, Fischer and coworkers correlated the configuration of the naturally occurring 1-*O*-alkylglycerols to D-(+)-glyceraldehyde whose configuration had arbitrarily been determined (Scheme 3). They assumed that 1-*O*-alkylglycerol can be formed by chemical interconversion of 1-*O*-alkyl-D-glyceraldehyde, without severing the bonds to the stereogenic carbon atom, by reduction. Since, 1-*O*-alkyl-D-glyceraldehyde is in turn homofacial with D-(+)-glyceraldehyde, this implying that the naturally occurring 1-*O*-alkylglycerols belong to the D-series and therefore exhibit the configuration shown in Scheme 3 (Baer and Fischer, 1939b).

The first empirical determination of the absolute configuration of any chiral compound was accomplished by Bijvoet and coworkers in 1951 on sodium rubidium (+)-tartrate tetrahydrate (Bijvoet et al., 1951). This established that (+)-tartaric acid had the absolute configuration *R*,*R*. (*R*,*R*)-(+)-tartaric acid has been correlated with many other chiral molecules, including D-(+)-glyceraldehyde, thus determining its *R* configuration. In this manner, the absolute configuration of the naturally occurring 1-*O*-alkylglycerols as related from (*R*)-(+)-glyceraldehyde was confirmed to be *S* (Scheme 3).

Racemic 1-*O*-alkylglycerols have been resolved into their enantiomers by high-performance liquid chromatography (HPLC). The separation of enantiomers from (*rac*)-chimyl alcohol as their corresponding diastereomeric bis-(*S*)-1-(1-naphthyl)ethylcarbamates on HPLC has been reported (Michelsen et al., 1985). Several reports have described the resolution of racemic 1-*O*-alkylglyerols as their bis-(3,5-dinitrophenylurethane) derivatives by chiral-phase HPLC (Itabashi and Tsuda, 2000; Takagi and Itabashi, 1986; Yamashina et al., 2006).

Lipase kinetic resolution has also been employed to resolve racemic batyl, chimyl and selachyl alcohols by sequential lipase catalyzed transesterification using vinyl acetate as an acyl donor. During the transesterification process, the *Pseudomonas fluorescens* lipase began displaying high regioselectivity toward the end-position of the 1-*O*-alkylglycerols, but offering poor



Scheme 3. Correlation of naturally occurring 1-*O*-alkylglycerols with D-(+)-glyceraldehyde.

enantioselectivity. On the other hand, the lipase in its second subsequent acylation firmly discriminated between the 3-monoacetyl antipodes by preferring the (*S*)-1-*O*-alkyl-3-acetyl-glycerols, that were rapidly converted into their diacylated derivatives, leaving the (*R*)-1-*O*-alkyl-3-acetyl-glycerols almost unreacted (Halldorsson et al., 2004). The saturated (*S*)-batyl and (*S*)-chimyl alcohols were obtained in ≥95% enantiomeric excess (ee) and the monounsaturated (*S*)-selachyl alcohol in 93% ee as was established on their bis-(*S*)-1-(1-naphthyl)ethylcarbamate derivatives by HPLC. In addition, the *P. fluorescens* lipase has also been reported to resolve the 3-monotosylate of racemic chimyl alcohol by acylation with palmitic acid anhydride (Chenevert and Gagnon, 1993).

2.5. Biological effects

2.5.1. Biological effects

For centuries people in Scandinavian countries have used shark liver oil as a staple remedy for wound healing (Solomon et al., 1997). For instance, in Iceland Greenland shark liver oil has been used to battle gastric ulcers, colon inflammation, scrofula (a disease which causes inflammation of lymphatic glands) and arthritis. Furthermore, in Iceland it has been a putative belief that shark liver oil has beneficial health effects on humans and to be prophylactic against all kinds of illness (Gisladottir, 1999).

Brohult et al. were the first to demonstrate that alkylglycerols isolated from Greenland shark liver oil were responsible for the positive health effects of shark liver oil on patients with cancer of uterine cervix undergoing radiation treatment (Hallgren, 1983). To some extent the treatment with alkylglycerols or shark liver oil prevented leucopenia and thrombocytopenia in those patients who received alkylglycerols or their diesters orally prior to radiation treatment. Moreover, mortality was lower in patients treated prophylactically with alkylglycerols, resulting in an increased survival rate compared to controls.

Further studies on the adjuvant and immunostimulant properties of alkylglycerols have been reported and among them a study from Ngwenya and Foster (1991), where 1-*O*-dodecylglycerol was tested for adjuvant and antibody response. Their results showed, that when mice were treated with 1-*O*-dodecylglycerol and immunized with sheep erythrocytes the mice developed a greatly enhanced antibody production. Yamamoto and Ngwenya have indeed reported the *in vivo* effect of 1-*O*-dodecyglycerol on macrophage stimulation (Yamamoto and Ngwenya, 1987). 1-*O*-dodecylglycerol was found to be the most potent macrophage stimulator compared to lysophospholipids and their alkyl analogues *in vivo*. Interestingly, trace amounts of 1-*O*-dodecylglycerol (0.05 μg/ml) were enough to induce a markedly elevated ingestion activity of macrophages. On the other hand, higher amounts of 1-*O*-dodecylglycerol were encountered to be quite toxic to the host immune system and even higher amounts provoked immune depression.

A study on the possible pathway involved in alkylglycerol stimulation of machophages by Yamamoto et al. concluded that 1-*O*-dodecylglycerol initiates the stimulation by acting on B-cells, which then transmit a signal factor to T-cells, that in turn modify the factor or produce a new one capable of activating a macrophage and induce phagocytosis (Yamamoto et al., 1988). Recently, synthetic alkylglycerols possessing 16, 12 and 10 carbon atom alkyl chains, presumably as racemates, were assayed for their adjuvant capability on the standardized antigen Ova (a mix of ovoalbumin), showing that alkylglycerols increased the anti-Ova IgG1 and IgG2a antibody production in sera of immunized mice *in vivo*. These results indicate that 1-*O*-alkylglycerols are effective adjuvants for the antigen Ova (Acevedo et al., 2006).

Pedrono et al. have investigated the effect of alkylglycerols (isolated from shark liver oil) and shark liver oil (containing about 25% DAGE and deprived of squalene) on tumour growth, pulmonary metastasis and vascularisation of Lewis lung carcinoma (3LL) cells grafted in mice (Pedrono et al., 2004). The results showed that both the shark liver oil and the mixture of natural alkylglycerols reduced the 3LL-grafted carcinoma up to similar percentages, 29 and 26%, respectively, compared to controls. Shark liver oil treatment induced 31% decrease of pulmonary metastasis number and alkylglycerols curtailed up to 64% the metastasis rate in grafted mice. Interestingly enough, the alkylglycerol treatment induced a significant reduction of the tumour blood vessels endothelial marker, therefore demonstrating that the antineoplastic effect of alkylglycerols is at least in part due to their antiangiogenic activity on tumour cells. Recently, the same scientific group has demonstrated that the monounsaturated C18:1n-9 (selachyl alcohol) and C16:1n-7 1-*O*-alkyl-*sn*-glycerols exerted the highest tumour growth depression and lowering of metastasis number in the mice grafted with 3LL cells, compared to the saturated C16:0 and C12:0 1-*O*-alkyl-*sn*-glycerols (Deniau et al., 2010). Strong tumour growth inhibition of three prostate cancer cells (DU-145, PC-3 and PCa-2b) by shark liver oil, presumably owing to the presence of alkylglycerols as DAGE, has also been reported *in vitro* (Krotkiewski et al., 2003).

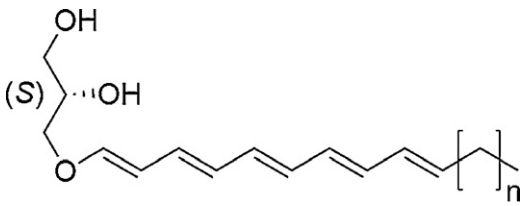
1-*O*-dodecyl-*rac*-glycerol has exhibited antibacterial activity against several different strains of bacteria. It's minimum inhibitory concentration is 4 μg/ml compared to 9 μg/ml, for its ester analogue, when tested on *Streptococcus faecium* ATCC 9790. In *S. faecium* the antibacterial activity mechanism of 1-*O*-dodecyl-*rac*-glycerol involves stimulation of peptidoglycan hydrolase (autolysin), probably via activation of a proteinase as an intermediary. It is noteworthy, that antibacterial activity of alkylglycerols increases with longer 1-*O*-alkyl chains from octanyl to dodecanyl, but decreases with longer alkyl chains (Ved et al., 1984). The antifungal activities of racemic 1-*O*-dodecylglycerol have indeed been reported along with its synergism with the strong antifungal agent amphotericin B (Haynes et al., 1994). A rather uncommon alkylglycerol 1-*O*-tridecyl-*sn*-glycerol, isolated from an unidentified marine sponge species has been reported to display toxicity to goldfish (Myers and Crews, 1983).

Fecapentaenes are conjugated polyunsaturated glyceryl ethers found in human feces, which display strong mutagenic properties and are suspected of being colon carcinogens. As can be seen for **9** and **10** in Fig. 6 they are *sn*-1 alkylglycerols and possess the *S* configuration (Hirai et al., 1985). Interestingly, other types of conjugated polyunsaturated alkylglycerols have been reported from the sponges *Raspailia pumila* and *ramosa* from the North-East Atlantic (Guella et al., 1987).

1-*O*-alkylglycerols have been shown to increase the permeability of the blood-brain barrier (BBB) facilitating the delivery of antibiotics and antineoplastic drugs to the brain (Gopinath et al., 2002). Intra-arterial coadministration of the antineoplastic drug methotrexate with 1-*O*-pentyl-*rac*-glycerol to C6 rat astroglioma bearing rats greatly increased the delivery of methotrexate in the tumour brain and the surrounding brain tissue (Erdlenbruch et al., 2000). Further studies by Erdlenbruch et al. (2003) suggest the enhanced permeability of the BBB by *rac*-alkylglycerols to bee elicited via increased opening of the tight junctions between cells constituting BBB.

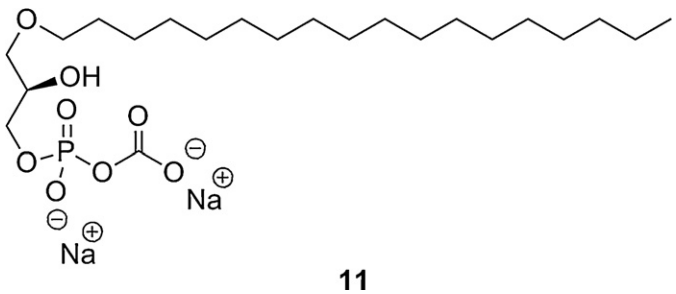
In vitro incubation of boar spermatozoa with a mixture of 1-*O*-alkyl-*sn*-glycerols isolated from shark liver oil increased spermatozoa motility and fertility in artificial inseminations of breeding sows, leading to increased number of farrows. Alkylglycerol treatment increased the production of lyso-PAF, which may display an agonist effect on PAF-receptors inducing spermatozoa motility and fertility (Cheminade et al., 2002).

Alkylglycerol derivatives comprising the antiretroviral drug phosphoformic acid at the *sn*-3 position of the glycerol moiety **11** (Fig. 7) are designed prodrugs, which have shown potent inhibition toward both wild type HIV-1 and most NRTI (nucleoside reverse transcriptase inhibitor)-resistant variants *in vitro*. Such alkylglycerol phosphoformate prodrugs have the advantage of possessing a greater bioavailability than unmodified phosphoformate presumably, by decreasing its charge at physiological pH from −3 to −2 and making it more lipophilic facilitating cellular uptake from the gastrointestinal tract (Hammond et al., 2001).



9 (n = 1)
10 (n = 3)

Fig. 6. Structure of fecapentaenes.



11

Fig. 7. An alkylglycerol prodrug disodium 1-*O*-octadecyl-*sn*-glycero-3-phosphoformate **11**.

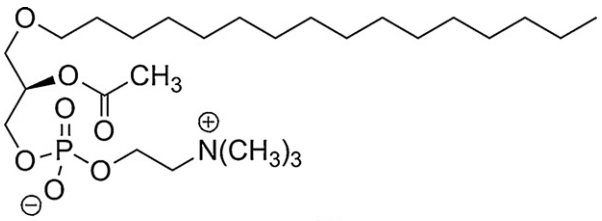
2.5.2. Alkylglycerols affect protein kinase C

Protein kinase C (PKC) plays a pivotal role in mediating cellular responses to extracellular stimuli, i.e. signal transduction. It's function is of high relevance for a variety of cell processes including regulation of cellular adhesion, cell growth and differentiation (Dekker and Parker, 1994; Nishizuka, 1986). Since a direct correlation was found between the ability of phorbol esters to selectively activate PKC and to induce tumourigenesis, some PKC isozymes have been suggested to play an important role in tumour promotion (Nishizuka, 1984). High PKC levels have been reported in tumour cell lines from breast, lung and of gastric origin (Mackay and Twelves, 2003), therefore, suggesting that some PKC isoforms are potential targets in the treatment for some types of cancer (Mackay and Twelves, 2003).

PKC is a family of serine/threonine kinases composed of at least 12 isoforms, whose function is to activate via phosphorylation selected proteins involved in different cell processes. The activation of a specific receptor by an extracellular signalling molecule is coupled to the activation of phospholipase C. Phospholipase C, in turn, hydrolyses the membrane-bound phosphatidylinositol-4,5-bisphosphate, resulting in the generation of two second messengers, i.e. 1,2-diacylglycerol (DAG), which remains embedded in the cell membrane, and inositol-1,4,5-triphosphate (IP_3), which is dissolved in the cytosol. IP_3 stimulates the release of Ca^{2+} ions from endoplasmic reticulum into cytosol, therefore triggering the translocation of inactive cytosolic PKC to the cytoplasmic face of a membrane fraction, where it is activated by DAG (Alberts et al., 1994). DAG plays a central role in PKC regulation during cell growth and it has been demonstrated that its concentration is elevated in proliferating cells (Warne et al., 1995). Alkylglycerols are inhibitors of PKC and are, therefore, antagonists of PKC in contrast to DAG and phorbol esters, which are PKC agonists.

Alkylglycerol induced PKC inhibition has been demonstrated in cell cultures of Madin-Darby canine kidney (MDCK), where 1-*O*-dodecyl-*sn*-glycerol was observed to reduce PKC activity in unstimulated cells and to inhibit the activation of PKC by phorbol esters. Furthermore, the content of alkylglycerol was highly regulated during cell growth and accumulated in growth-inhibited MDCK cells (Warne et al., 1995), therefore, suggesting that endogenous alkylglycerols are possibly used to regulate cell growth by inhibition of PKC.

The inhibition properties of alkylglycerols have also been demonstrated on isolated PKC with 1-*O*-dodecyl-*rac*-glycerol, 1-*O*-hexadecyl-*rac*-glycerol, 1-*O*-octadecyl-*rac*-glycerol and a mixture of naturally occurring alkylglycerols comprising 1-*O*-alkyl chains of 22 and 24 carbon atoms isolated from *L. donovani* (McNeely et al., 1989; Warne et al., 1995). Interestingly, the acyl analogue of (*rac*)-chimyl alcohol, i.e. 1-monopalmitoyl-*rac*-glycerol, has been shown to exhibit only low PKC inhibition, and the diether 1,2-di-*O*-hexadecyl-*rac*-glycerol had no effect on PKC activity (McNeely et al., 1989). The 1-*O*-alkyl-*sn*-glycerol derivatives 1-*O*-alkyl-2-acetyl-*sn*-glycerols containing palmityl and oleyl alkyl chains,



12

Fig. 8. A PAF molecule 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phophocholine **12**.

1-*O*-hexadecyl-2-*O*-methyl-*rac*-glycerol and 1-*O*-hexadecyl-2-*O*-ethyl-*rac*-glycerol have likewise been observed to inhibit PKC (Daniel et al., 1988). These results indicate that the ether function is essential for inhibition activity on PKC, regardless of whether the 1-*O*-alkyl moiety is situated at the *sn*-1 or *sn*-3 position of the glycerol backbone.

Regarding the DAG analogues, 1-*O*-alkyl-2-acetyl-*sn*-glycerols as inhibitors of PKC raises the question about a probable anti-cancer effect of alkylglycerols, not only by direct PKC inhibition, but by their first incorporation into plasmanyl-phospholipids in cell membranes which release the DAG analogues by the action of phospholipase C. Such incorporation of alkylglycerols into plasmanyl-phospholipids, serving as precursors of the PKC inhibitors, 1-*O*-alkyl-2-acetyl-*sn*-glycerols has been demonstrated in porcine aortic endothelial cells (Marigny et al., 2002).

We can infer that the anticancer properties of alkylglycerols might be mediated by inhibition of some PKC isoforms in tumour cells, since various PKC isozymes are clearly involved in tumour promotion. Indeed, a recent study revealed that downregulation of PKC-β isoform hampered tumour development in endothelial cells via strong inhibition of angiogenesis in mice (Yoshiji et al., 1999), thus, denoting the possible antineoplastic action of alkylglycerol via PKC inhibition implicated in reduced tumour vascularisation. Remarkably, alkylglycerols have been reported to be responsible for both the anti-tumour and anti-angiogenic properties of shark liver oil in Lewis lung carcinoma cells in mice (Pedrono et al., 2004). In addition, the same study showed that alkylglycerols increased the permeability of endothelial cells, presumably by changes in the cytoskeleton and cell-to-cell adhesion, that could be involved in tumour growth inhibition.

2.5.3. Alkylglycerols and PAF

Since the discovery of PAF nearly 40 years ago (Benveniste et al., 1972) it has emerged as one of the most important lipid mediators known. The structure of PAF has been identified as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. In nature PAF includes several molecular species, differing by their hydrocarbon chain length and/or unsaturation at the *sn*-1 position of the glycerol backbone (Fig. 8).

PAF is a multifunctional cellular mediator found in various cell types and systems. It is involved in various human pathophysiological processes, such as platelet activation, asthma, allergy, ischemia and shock (Centemeri et al., 1999). PAF is involved in all pro-inflammatory reactions (Snyder, 1999) and plays an important role in neuronal functions and reproduction, including sperm fertility, ovulation, fertilization, pre-implantation, embryo development, implantation and parturition in mammals (Cheminade et al., 2002; Koltai et al., 1991; Roudebush et al., 2005). PAF acts on various cell types such as human platelets, macrophages and spermatozoa through specific PAF-receptors which are coupled to several transduction systems. Activation of PAF-receptors on spermatozoa stimulates their motility, capacitation and acrosome reaction in humans, mice and rabbits (Cheminade et al., 2002). PAF binds to PAF-receptors, which are usually on the cell surface, and elicit production of IP_3, DAG and increasing concentrations of calcium ions, which regulate nicotinamide adenine dinucleotide (NAD) kinase activity and a cascade of subsequent events, which end in a final response (Roudebush et al., 2005). The endogenous synthesis of PAF begins with the hydrolysis of the *sn*-2 position of 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine by phospholipase A_2 forming lyso-PAF (1-*O*-alkyl-*sn*-glycero-3-phosphocholine), which is then acetylated by lyso-PAF-acetyl transferase with acetyl-CoA as the acylating agent to produce PAF. The PAF-acetylhydrolase's role is to deactivate PAF when necessary via hydrolysis of the *sn*-2 position, reproducing lyso-PAF (Roudebush et al., 2005).

In searching for answers about a possible pathway by which 1-*O*-alkylglycerols exhibit their numerous biological activities in mammals, many scientists have proposed their implication in PAF synthesis by acting as a PAF precursor. Exogenous 1-*O*-alkyl-*sn*-glycero-3-phosphocholines have been shown to increase the PAF production in human neutrophils and macrophages (Dentan et al., 1996; Jouvin-Marche et al., 1984). In this regard, Hichami et al. (1997) have demonstrated the *in vitro* incorporation of a mixture of labelled naturally occurring 1-*O*-alkyl-*sn*-glycerols, isolated from shark liver oil, into the 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholines, which were subsequently used for the synthesis of PAF analogues (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines) in human leukemic monocyte-like THP-1 cells. Furthermore, alkylglycerol incorporation resulted in a significant rise in PAF production by THP-1 cells under resting and stimulated conditions. Therefore, it may be concluded that 1-*O*-alkyl-*sn*-glycerols may induce their biological activities by increasing the pool of PAF precursors in cells.

2.6. Alkylglycerol uptake in mammals

Dietary 1-*O*-alkylglycerols supplements, as diols, diacyl derivatives or ether phospholipids, are well tolerated by humans and animals (Mangold, 1983; Paltauf, 1972). Presumably in the gut the ester moieties are hydrolyzed, releasing the 1-*O*-alkylglycerols. Studies on the absorption of dietary alkylglycerols in mammals, including humans, have revealed that both enantiomers, *sn*-1 and *sn*-3 alkylglycerols, are completely absorbed by the intestine, without hydrolysis of the ether linkage (Bergstrom and Blomstrand, 1956).

It has been shown that after absorption only the natural 1-*O*-alkyl-*sn*-glycerols are incorporated into lipids of different tissues, while the 3-*O*-alkyl-*sn*-glycerol antipodes are primarily oxidized to the corresponding fatty acids (Das et al., 1992; Paltauf, 1971). 1-*O*-Alkyl-*sn*-glycerols are incorporated into the diacyl glyceryl ethers and ether phospholipids, ethanolamine and choline derivatives of plasmanyl- and plasmenyl-phospholipids, of lipid tissues (Reichwald and Mangold, 1977). Indeed, one study showed that 1-*O*-alkyl-*sn*-glycerols bearing fairly shorter alkyl chains are incorporated to a small extent into phospholipids and that only the long saturated or monounsaturated 1-*O*-alkyl chains are harness for the biosynthesis of plasmalogens (Blank et al., 1991).

It has been demonstrated that following 1-*O*-alkyl-*sn*-glycerol oral administration the concentration of ether-linked glycerolipids, including DAGE and ether phospholipids, is substantially increased compared to controls. Such increase was offset by decrease in the fatty esters subclass in the tissues investigated (Blank et al., 1991). Even though incorporation of dietary 1-*O*-alkyl-*sn*-glycerols has been demonstrated into plasmalogens of all tissues, their incorporation into brain tissue has only been reported to a very small extent in rats (Das and Hajra, 1988; Das et al., 1992).

Further studies on the fate of 1-*O*-alkyl-*sn*-glycerols administrated orally to pregnant rats showed that alkylglycerols were transferred from mother to suckling rats, but not from pregnant rats to foetuses (Das et al., 1992). How exactly exogenous

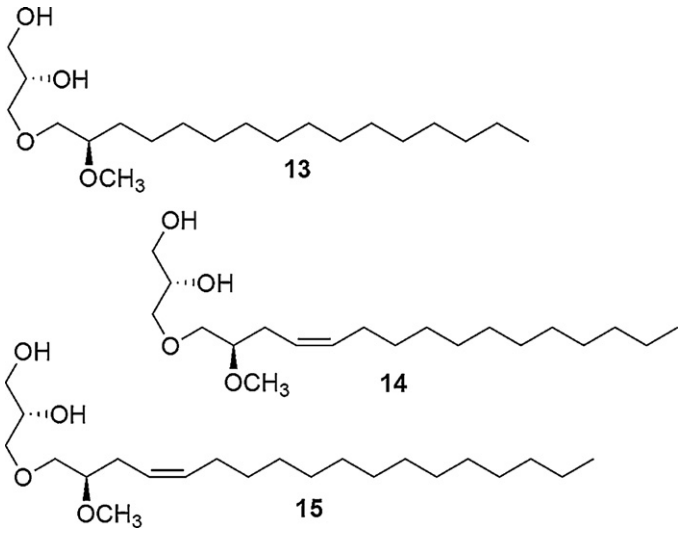


Fig. 9. The major 2′-methoxylated alkylglycerols in cartilaginous fish liver oil.

alkylglycerols are transformed to their corresponding neutral and phospholipid derivatives in supplemented animals is not known, but it is plausible that they enter the ether lipid biosynthetic pathway at two different stages (Scheme 1). They might enter following phosphorylation of their *sn*-3 position, forming 1-*O*-alkyl-*sn*-glycero-3-phosphates, or they might enter following esterification with acyl-CoA at their *sn*-2 position, forming 1-*O*-alkyl-2-acyl-*sn*-glycerols. In both cases these derivatives are finally converted to their corresponding neutral phospholipids or ether phospholipids and transported from intestinal mucosa to other tissues by a delivery system yet unknown.

3. 1-*O*-(2′-methoxyalkyl)-*sn*-glycerols

Methoxyl substituted alkylglycerols of the (2′*R*)-1-*O*-(2′-methoxyalkyl)-*sn*-glycerol type are naturally occurring *sn*-1 glyceryl ethers. They are characterized by possessing a methoxyl group at the second carbon of the 1-*O*-alkyl chain. They were discovered and isolated for the first time by Hallgren and Stallberg in 1967 from the unsaponifiable matter of Greenland shark (*Somniousus microcephalus*) liver oil, where they accounted for 4% of the total amount of the glyceryl ethers (Hallgren and Stallberg, 1967). The principal constituents of the methoxylated alkylglycerol fraction were found to be a saturated 1-*O*-(2′-methoxyhexadecyl)-*sn*-glycerol **13** and two monounsaturated analogous (*Z*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol **14** and (*Z*)-1-*O*-(2′-methoxyoctadec-4′-enyl)-*sn*-glycerol **15**, comprising a *Z*-configured double bond at the 4-position of the alkyl moiety (Fig. 9).

Some years later Hallgren et al. (1971) found a remarkable docosahexaenoic acid (DHA) like methoxylated alkylglycerol, all *cis* 1-*O*-(2′-methoxydocosa-4′,7′,10′,13′,16′,19′-hexaenyl)-*sn*-glycerol **16** (Fig. 10), also from Greenland shark liver oil. More recently the C16:0 and C16:1 methoxyl substituted alkylglycerols have been reported from the brachiopod *Gryphus vitreus* (D'Ambrosio et al., 1996). The absolute configuration of these peculiar alkylglycerol analogues was unknown for two decades after their discovery, until 1990, when Stallberg determined the *R* configuration at the carbon bearing the methoxyl group in the alkyl chain and the *S* configuration at the mid-positioned carbon atom of the glycerol moiety, implying their *sn*-1 configuration like the ordinary 1-*O*-alkyl-*sn*-glycerols and 1-*O*-(alk-1′-enyl)-*sn*-glycerol analogues (Stallberg, 1990). This was achieved by comparing the ^{1}H NMR spectra and optical activities of the four synthetic diastereomers of 1-*O*-(2′-methoxyhexadecyl)glycerol with those of the natural product.

3.1. Occurrence

The presence of 2′-methoxylated alkylglycerols has been reported in cartilaginous fish, marine animals and in terrestrial mammals including humans. Hayashi and Takagi (1982) investigated the methoxylated alkylglycerols from the liver oil of six cartilaginous fish species, including three sharks, *Scyliorhinus torazame*, *Squalus acanthias* and *Dalatias licha*, and three ratfish species, *Hydrolagus novaezealandiea*, *H. barbouri* and *Rhinochimaera pacifica*. They reported the methoxylated alkylglycerol content in the liver lipids to range from 0.04% in the shallow-water shark *S. torazame* to 0.3% in the deep-sea chimaera *H. barbouri*. In the liver lipids of the ratfish the methoxylated alkylglycerols accounted for 0.4–1.3% of the total glyceryl ether content, but higher percentages were found in the sharks, 2.2, 2.5 and 14.8%, in *S. acanthias*, *D. licha* and *S. torazame*, respectively. In this regard, our studies have shown that a mixture of shark liver oils obtained from certain deep-sea sharks species from the North Atlantic exhibit high percentages of methoxylated alkylglycerols, 1.46% of the oil and 7.0% of the glyceryl ether content (Unpublished results).

Hallgren et al. (1974a) reported the percentages of methoxylated alkylglycerols in the neutral lipids and phospholipids of certain aquatic animal species. In that study the percentages of methoxylated alkylglycerols in herring fillets, Baltic herring, mackerel fillets, marine crayfish, fresh-water crayfish, shrimps, sea mussels, commercial cod liver oil and Baltic cod liver oil ranged from 0.01 to 0.07% in the neutral fat, while those of phospholipids ranged from 0.06 to 0.47%. The concentration of methoxylated alkylglycerols was higher in the phospholipids than in the neutral lipids of all the species investigated except in fresh-water crayfish, where the levels were similar. The methoxylated alkylglycerol amounts were 7–14 times higher in the phospholipids than in the neutral lipids of the teleost fish, and 2–6 times higher in the invertebrates. The amount of methoxylated alkylglycerols within the glyceryl ethers were especially high in the neutral lipids of herring fillet, mackerel fillet, commercial cod liver oil and Baltic cod liver oil, 28, 25, 37 and 28%, respectively, and moderately high in Baltic herring, marine crayfish, fresh-water crayfish, shrimps and sea mussels, 12, 12, 10, 6, and 7%, respectively. Likewise, in

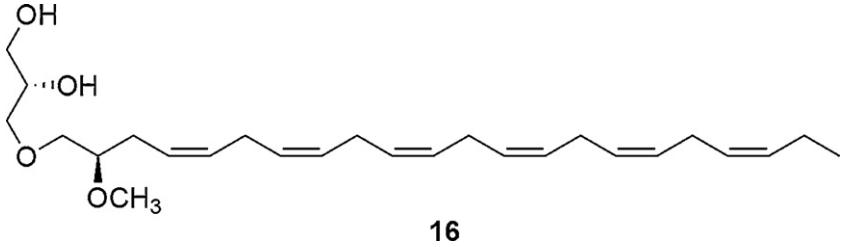


Fig. 10. A DHA like 2′-methoxylated alkylglycerol **16**.

the phospholipids such percentages were high in herring fillets, Baltic fillets, mackerel fillets, marine crayfish, shrimps and sea mussels, 20, 20, 17, 21, 10 and 20%, respectively, and relatively low in the fresh-water crayfish, 3%. Interestingly, the percentages of the methoxyl substituted alkylglycerols within the glyceryl ethers were higher in the marine species studied, than in the liver oil of cartilaginous fish reported by Hallgren and Stallberg (1967) and Hayashi and Takagi (1982).

Hallgren's group investigated the occurrence of methoxylated alkylglycerols isolated from the non-polar and phosphorylated lipids of human colostrum, human milk, cow milk, sheep milk, human red bone marrow, red cells, blood plasma and uterine carcinoma. They found the methoxyl substituted glyceryl ethers in only trace quantities, both in the neutral lipids and phospholipids of all the tissues examined (Hallgren et al., 1974b).

It can be concluded that 1-*O*-(2′-methoxyalkyl)-*sn*-glycerols are usually more prevalent in the phospholipids than in the neutral lipids of marine animal. In addition, their content is higher in marine animals than in terrestrial mammals, both in the neutral and especially in the phospholipid fractions (Hallgren et al., 1974b).

3.2. Biosynthesis and 1-O-(2′-methoxyalkyl) chain composition

The 1-*O*-(2′-methoxyalkyl) chain compositions of methoxyl substituted alkylglycerols have been studied thoroughly in the liver oil of cartilaginous fish, various marine animals and mammals. When the results reported by Hayashi and Takagi (1982) of three shark species and three chimaeras and that of the Greenland shark reported by Hallgren and Stallberg (1967) are compared they reveal that the long alkyl chains of the methoxylated alkylglycerols range from 14 to 22 carbon atoms. Furthermore, the methoxylated alkylglycerol possessing the Δ4-hexadecenyl chain is the most abundant adduct found in the liver oil of the cartilaginous fish, accounting for 31–60% of the methoxylated alkyl chains. The hexadecyl and octadec-4′-enyl chains amounted to roughly 15–30% and 6–20% of the methoxylated alkylglycerols, respectively. The octadecyl chain was found in high quantities (about 15%) in the liver oil of *H. Novaezealandiae*, but only in low amounts (about 3%) in other species. The 16 and 18 carbon atoms long 2′-methoxyalkyl chains together composed about 80% of the methoxylated alkylglycerols in the liver oil of the cartilaginous fish. The occurrence of the unusual polyunsaturated methoxyl substituted docosahexaenyl alkylglycerols was low in *S. somniosus* (>4%), high in *R. pacifica* (18%), but in the other species amounted to about 8% of the methoxylated glyceryl ethers.

The 1-*O*-(2′-methoxyalkyl) compositions of methoxylated glyceryl ethers in various marine animals and mammals, principally man, has been investigated (Hallgren et al., 1974a,b). The long chain components of the methoxylated alkylglycerols ranged from 14 to 22 carbons like in chondrichthyans. The 16 carbon atom long alkyl chains were essential constituents of the methoxylated alkylglycerols in the fillets of herring and mackerel and the mollusc marine crayfish and shrimps, where their content was about 93%, both in non-polar lipids and phospholipids. The hexadecyl moiety was found in high amounts of both the neutral lipids and phospholipids of sea mussels (52 and 66%, respectively) and of cow milk (about 90%). The hexadec-4′-enyl, hexadecyl and octadec-4′-enyl chains were found in similar amounts (about 23% each) in the neutral lipids of human red bone marrow and in the phospholipids of human red blood cells. The hexadec-4′-enyl chain constituted half the total amount of the 1-*O*-(2′-methoxyalkyl)-*sn*-glycerols of phospholipids of human red bone marrow. The Baltic cod liver oil was very rich in octadec-4′-enyl chains, that made up 70% of the methoxylated alkylglycerols.

The DHA like methoxylated alkylglycerol was found in the phospholipids of human red blood cells, shrimps, in the neutral lipids of mackerel and in cod liver oil, accounting for 7.6, 0.4, 0.5, and 1.7% of the methoxylated mixture, respectively. Furthermore, it has also been found in mammalian tissue but in only trace amounts.

It is noteworthy, that variations in quantity of the major 1-*O*-(2′-methoxyalkyl)-*sn*-glycerols in neutral lipids and phospholipids of the fillets of herring and Baltic herring were observed indicating differences in their dietary intake, presumably owing to the two groups of herring being associated with different habitats. Albeit, generally the 1-*O*-(2′-methoxyalkyl) composition profiles are similar in both the non-polar lipids and phospholipids of the same organism, nevertheless, considerable differences can be found among the major components like in the fresh water crayfish, which contains very high amounts of hexadecyl chains (50%) and low amounts of octadec-4′-enyl chains (4%) in their neutral lipids, in contrast to 32% and 28%, respectively, in the phospholipids.

Recently, two phosphochloline substituted 2′-methoxylated alkylglycerols comprising hexadecyl and hexadec-4′-enyl chains have been isolated from the marine sponge *Spirastrella abata*. Their molecular structures were confirmed as 1-*O*-(2′-methoxyhexadecyl)-*sn*-glycero-3-phosphocholine and (*Z*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycero-3-phosphocholine (Alam et al., 2001). Interestingly enough, the 16 carbon atom alkyl chains also seem to predominate in sponges as in other animal species.

In summary, the long alkyl chains of 1-*O*-(2′-methoxyalkyl)-*sn*-glycerols in marine animals and terrestrial mammals range from C14 to C22. The long chain components are primarily saturated, monounsaturated and polyunsaturated. The monounsaturated chains are characterized by comprising a Δ4 *Z*-configured double bond as has been confirmed for the hexadec-4′-enyl, heptadec-4′-enyl and octadec-4′-enyl methoxylated alkylglycerols of cartilaginous fish liver oil. The principal 1-*O*-(2′-methoxyalkyl)-*sn*-glycerols found in nature comprise hexadec-4′-enyl, hexadecyl and octadec-4′-enyl alkyl chains, although in some cases the octadecyl chain can be found in moderate quantities. The hexadec-4′-enyl methoxylated alkylglycerol is clearly the most abundant methoxylated alkylglycerol found in the liver oil of cartilaginous fish. The polyunsaturated carbon chains are characteristic of the methoxyl substituted alkylglycerols of cartilaginous fish liver oil. In this regard, the octadecatrienyl (C18:3) and docosapentaenyl (C22:5) chains have only been reported in chondrichthyans and the docosahexaenyl (C22:6n-3) is found in relatively high amounts in cartilaginous fish compared to other animals. Interestingly enough, the docosahexaenyl alkyl chain also contains a Δ4 *cis*-configured double bond like the monounsaturated congeners. The position and configuration of the double bonds in the octadecatrienyl and docosapentaenyl glyceryl ethers have not been determined so far.

Comparison between the alkyl chain compositions of methoxyl substituted and unsubstituted alkylglycerols shows that in both types the 16 and 18 carbon atom saturated and monounsaturated alkyl chains predominate. A relevant difference is the characteristic monounsaturation at the 4-position (Δ4) of the methoxylated alkyl chains which is absent in the unsubstituted alkyl chains. In addition, polyunsaturated alkyl chains are more prevalent in the methoxyl substituted alkylglycerols, primarily among chondrichthyans, than in the unsubstituted alkylglycerols.

The biosynthesis of the alkyl chain moiety of the methoxylated alkylglycerols is not known, but in contrast, it is very well known for the unsubstituted analogues. A logical biosynthetic pathway would involve the 1-*O*-(2′-methoxyalkyl) moieties being derived from the alcohols via corresponding α-methoxylated fatty acids. α-Methoxylated fatty acids are widely found in marine sponges (Carballeira, 2002) and they share two important features with the 1-*O*-(2′-methoxyalkyl) moieties, i.e. both possess a methoxyl group at carbon 2 and the stereochemistry of the chiral center is *R*, implying a convergent biosynthetic origin.

Accordingly, it can be expected that the methoxylated fatty acids could be reduced by fatty acyl-CoA reductases to the corresponding methoxyl substituted fatty alcohol. In this light, reductases may be expected to be responsible for the narrow chain length range of the long alkyl chains composing the methoxylated glyceryl ethers, primarily preferring 16 and 18 carbon atoms long methoxylated fatty acids as substrates. This hypothesis might work out for the saturated 2-methoxylated 1-*O*-alkyl chains, since 2-methoxylated fatty acids, C14:0, C15:0, C16:0, C18:0, C19:0, C20:0, C21:0 and C22:0, have been found in the phospholipids of various marine sponges. Likewise, as mentioned before, methoxylated C16:0 and C16:1 alkylglycerols have been found in a sponge species as choline phospholipid derivatives (Alam et al., 2001; Carballeira, 2002).

However, the postulated hypothesis would not work for the Δ4 monounsaturated alkyl chains, since naturally occurring Δ4 unsaturated methoxyl substituted fatty acids have, so far, not been reported. On the other hand, trace quantities of 2′-hydroxyl substituted alkylglycerols have been isolated from the unsaponifiable fraction of shark liver oil (Hallgren et al., 1978). The most prominent compound found was the hydroxylated C16:1 alkylglycerol, in addition, C14:0, C14:1, C16:0 and C18:1 alkyl chains were also identified. This finding clearly shows that 2′-hydroxylated alkylglycerols are direct precursors of the methoxylated analogues, indicating that the methylation of the hydroxyl group is performed after the 1-*O*-alkyl chain has been incorporated into the glycerol backbone. The presence of a docosahexaenyl methoxylated chain confirms that Δ4 desaturation of the fatty acid precursors comes before hydroxylation. Δ4 unsaturation is not uncommon in nature, for instance, DHA, an essential fatty acid of cell membranes in various tissues, has been demonstrated to be the product of Δ4 desaturation of 22:5n-3 by a Δ4 desaturase from *Thraustochytrium* sp. More recently, Δ4 palmitic acid was produced from saturated palmitic acid by a Δ4 desaturase isolated from seeds of *Hedera helix*. Furthermore, Δ4 monounsaturated fatty acids have been detected in marine and fresh water sponges (Dembitsky et al., 2003; Rodkina et al., 2008).

α-Hydroxylated fatty acids are found widespread in animal tissues, including sponges and mammals, plants and microorganisms (yeasts) (Carballeira et al., 1992; Foulon et al., 2005; Jenske and Vetter, 2009). They possess the *R* configuration at their unsymmetric carbon atom (Vesonder et al., 1970) and the acyl chains are generally saturated or monounsaturated, although long polyunsaturated acyl chains are abundant in sphingomyelin compounds found in testes and spermatozoa of mammals (Robinson et al., 1992). In addition, fatty acid 2-hydroxylases have been identified in yeasts and in human brain (Alderson et al., 2006, 2004; Uchida et al., 2007). Based on these reports, a novel biosynthesis of methoxylated alkylglycerols is postulated, shown in Scheme 4. The biosynthetic pathway is shown for the C16:0, C16:1 and C22:6n-3 methoxylated alkylglycerols, and assumes that they originate from hexadecanoic acid, Δ4 hexadecenoic acid and DHA. Then, they are α-hydroxylated and the acid group subsequently reduced, forming a diol, which is incorporated into the 1-acyl-DHAP by a synthase in analogy to the ordinary alkylglycerol biosynthesis. 1,2-Alkanediols have been shown to serve as precursors of 2′-hydroxylated alkylglycerols in myelinating rat brains (Muramatsu and Schmid, 1971). The methylation of the hydroxyl group is one of the last steps in the biosynthesis, and the reaction might be influenced by the kind of chemical groups attached to the *sn*-2 and/or *sn*-3 position of the glycerol backbone, similar to what is observed in the Δ1 desaturation reaction of 1-*O*-alkyl to 1-*O*-(alk-1′-enyl). The high amounts of Δ4 unsaturation in the 1-*O*-(2′-methoxyalkyl) profiles could be explained by specificity of fatty acyl-CoA reductases toward α-hydroxylated Δ4 unsaturated fatty acids.

Interestingly, there is another possibility that both the hydroxylation and methylation steps take place directly on the 1-*O*-alkyl moieties, and such pathway cannot be ruled out. In that case a possible 1-*O*-alkyl substrate discrimination involved in the α-hydroxylation should not be discarded. Nevertheless, the biosynthetic pathway shown in Scheme 4 is considered to be built on more solid reaction sequences than the second one, especially regarding the fact that hydroxylation of fatty acids is a widespread reaction in nature. Furthermore, the hydroxylated fatty acids possess the *R* configuration, which is the same as found in the 2′-methoxylated alkylglycerols.

3.3. Chemical synthesis

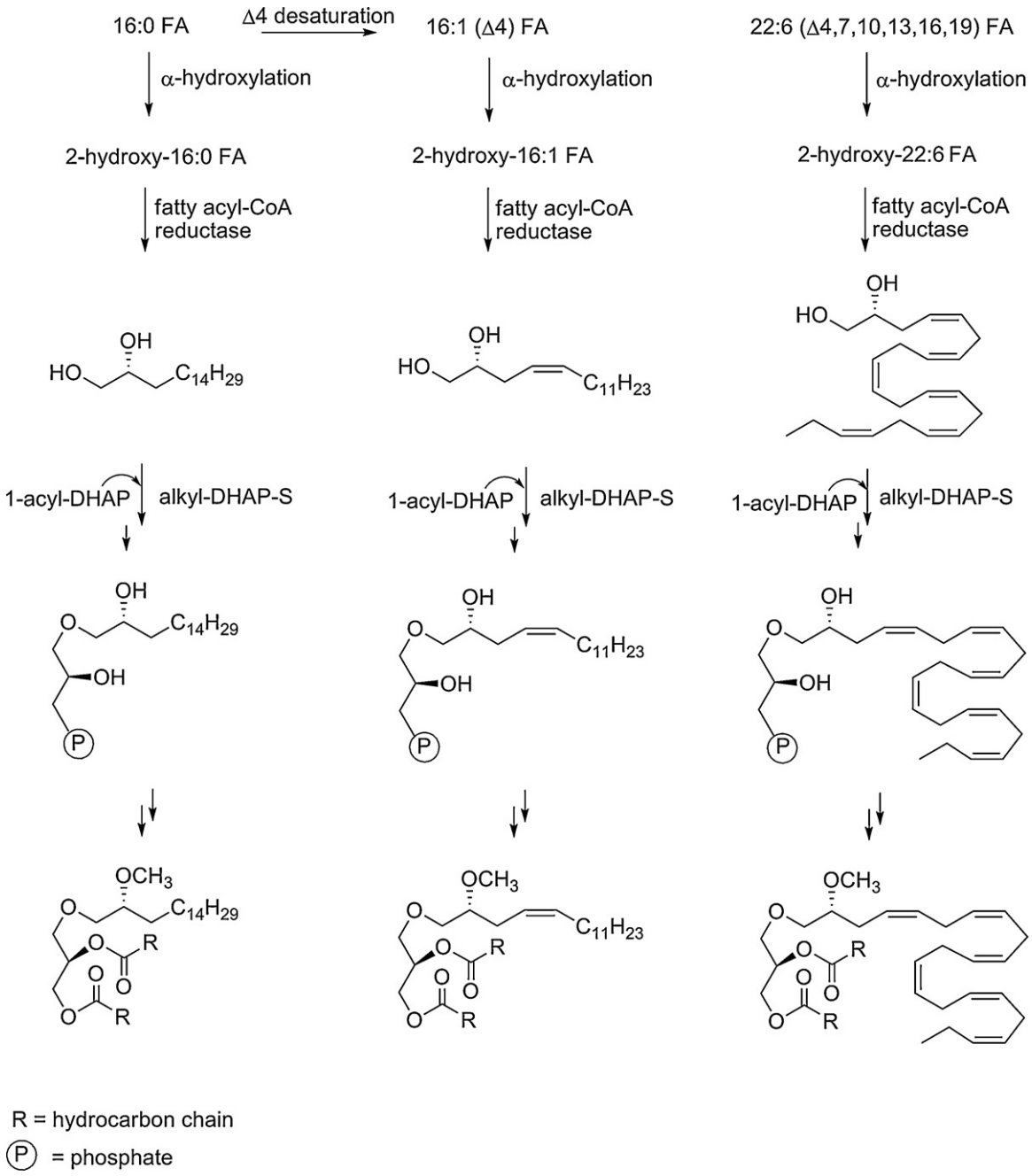
Hallgren and Stallberg (1967) reported the first synthesis of a methoxylated alkylglycerol 1-*O*-(2′-methoxyhexadecyl)glycerol **22** used for structural elucidation of naturally occurring methoxylated alkylglycerols, isolated from the unsaponifiable fraction of Greenland shark liver oil. The synthesis is shown in Scheme 5 and starts with the one-pot conversion of palmitic acid to methyl 2-bromohexadecanoate **17**, by treating palmitic acid with thionyl chloride. This was followed by α-bromination of the resulting palmitic acid chloride with bromine and, finally, methyl esterification by addition of methanol, in 81% yield. Subsequent displacement of the bromine atom by sodium methoxide in methanol solution afforded methyl 2-methoxyhexadecanoate **18** in 95% yields. Reduction of the ester with lithium aluminium hydride and subsequent treatment of the resulting alcohol **19** with *p*-toluenesulfonic acid chloride, afforded 2-methoxyhexadecyl *p*-toluenesulfonate **20** in 70% overall yield for the two steps.

Coupling of **20** with the potassium salt of (*rac*)-1,2-*O*-isopropylideneglycerol (40% mol excess), obtained via potassium in benzene, yielded 1-*O*-(2′-methoxyhexadecyl)-2,3-*O*-isopropylideneglycerol **21** in 45% yield. Finally, the isopropylidene moiety was hydrolyzed in 0.5 M HCl at 100 °C, yielding the 1-*O*-(2′-methoxyhexadecyl)glycerol **22** as a mixture of stereoisomers in 83% yield (20% overall yield starting from palmitic acid).

The way Hallgren and Stallberg approached the synthesis of the 2′-methoxylated alkylglycerols is similar to the already reviewed methods utilized for synthesis of the unsubstituted alkylglycerols, which are based on the coupling of potassium or sodium salt of 1,2-*O*-isopropylideneglycerol with a long alkyl chain, comprising a good leaving group at position 1. For the synthesis of substituted alkylglycerols it was therefore obvious to couple the acetone-protected glycerol with an appropriately substituted alkyl chain bearing a good leaving group at position 1, for example as alkyl *p*-tosylates.

Following the same methodology, Stallberg (1975) synthesized various substituted alkylglycerols, and like in the synthesis of 1-*O*-(2′-methoxyhexadecyl)glycerol, he employed substituted alkyl tosylates obtained via reduction of the corresponding acid derivatives. Among the substituted alkylglycerols that Stallberg synthesized was the monounsaturated 2′-methoxylated alkylglycerol (*Z*)-1-*O*-(2′-methoxyhexadec-4′-enyl)glycerol **28** obtained as a mixture of 4 stereoisomers (Scheme 6).

The synthesis was started with α-alkylation of methyl methoxyacetate enolate with 1-bromotetradec-2-yne, using potassium metal, affording methyl 2-methoxyhexadec-4-ynoate **23** in about 80% yield. The 2-methoxylated ester was then reduced by Red-Al in hexane to the corresponding alcohol **24**, which was subsequently converted to the 2-methoxyhexadec-4-ynyl *p*-toluenesulfonate **25** in presence of *p*-toluenesulfonic acid chloride and pyridine. Adduct **25** was then coupled with (*rac*)-1,2-*O*-isopropylideneglycerol in the presence of potassium hydroxide in *n*-heptane, affording 1-*O*-(2′-methoxyhexadec-4′-ynyl)-2,3-*O*-isopropylideneglycerol **26** in 65% yield. Partial hydrogenation of the acetylenic compound with 5% palladium on barium sulphate as catalyst in



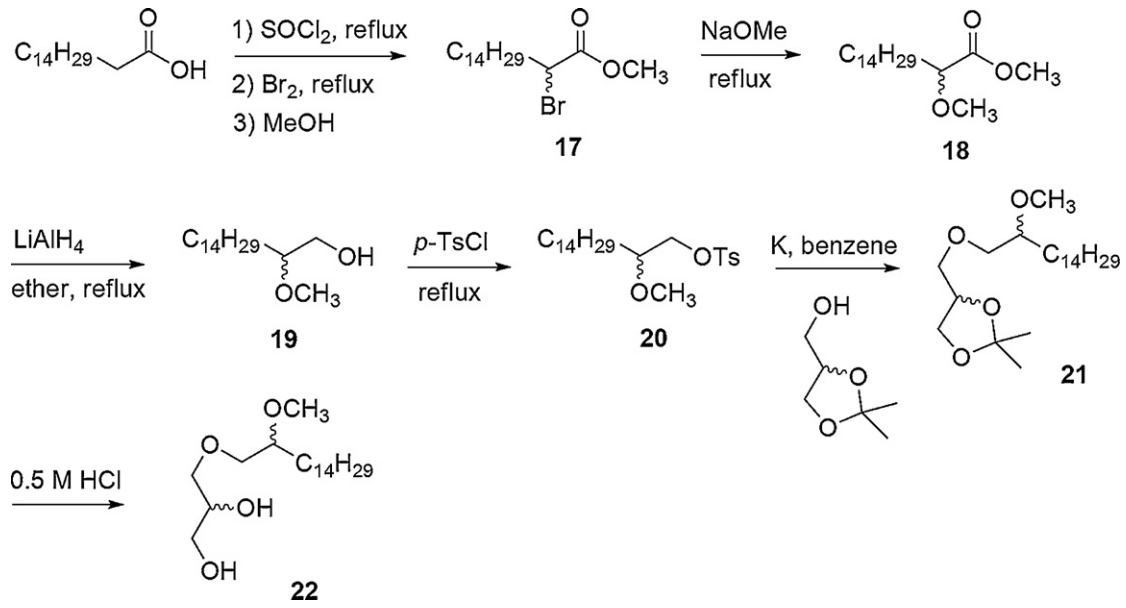
Scheme 4. Postulated biosynthesis of 2′-methoxylated alkylglycerols diesters.

pyridine furnished (*Z*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-2,3-*O*-isopropylideneglycerol **27**, comprising the *cis*-configured double bond in 83% yield. Finally, hydrolysis of the acetonide with a mixture of ether and hydrochloric acid at 5 °C afforded the desired (*Z*)-1-*O*-(2′-methoxyhexadec-4′-enyl)glycerol **28** in 78% yield.

Stallberg (1990) performed the first synthesis of the naturally occurring (2′*R*)-1-*O*-(2′-methoxyhexadecyl)-*sn*-glycerol enantiomerically pure via the two routes shown in Scheme 7 and **8**. There, the chiral precursors (*S*)-1-benzyloxy-2,3-epoxypropane and (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol were exploited.

Both pathways are based on the prior synthesis of (*R*)-2-methoxyhexadecan-1-ol **30**, which was achieved by the copper-catalyzed reaction of tridecylmagnesium bromide with (*S*)-1-benzyloxy-2,3-epoxypropane, subsequent *in situ* methylation with dimethylsulfate, and cleavage of the benzyl group by hydrogenolysis, yielding the 2-methoxylated fatty alcohol intermediate in 68% yield. The first route (Scheme 7) proceeds with the coupling of (*R*)-2-methoxyhexadecan-1-ol **30** with (*S*)-1-benzyloxy-2,3-epoxypropane in the presence of sodium hydride in dimethylformamide (DMF), affording (2′*R*)-1-*O*-(2′-methoxyhexadecyl)-3-*O*-benzyl-*sn*-glycerol **31** in 43% yield. Finally the benzyl group was cleaved by hydrogenolysis of **31**, affording (2′*R*)-1-*O*-(2′-methoxyhexadecyl)-*sn*-glycerol **13** in 29% overall yield.

The second route (Scheme 8) starts with tosylation of **30**, providing (*R*)-2-methoxyhexadecyl *p*-toluenesulfonate **32** in 93% yields. Adduct **32** was coupled with (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol in the presence of powdered potassium hydroxide in *n*-heptane giving the isopropylidene condensation derivative **33** in 60% yields.

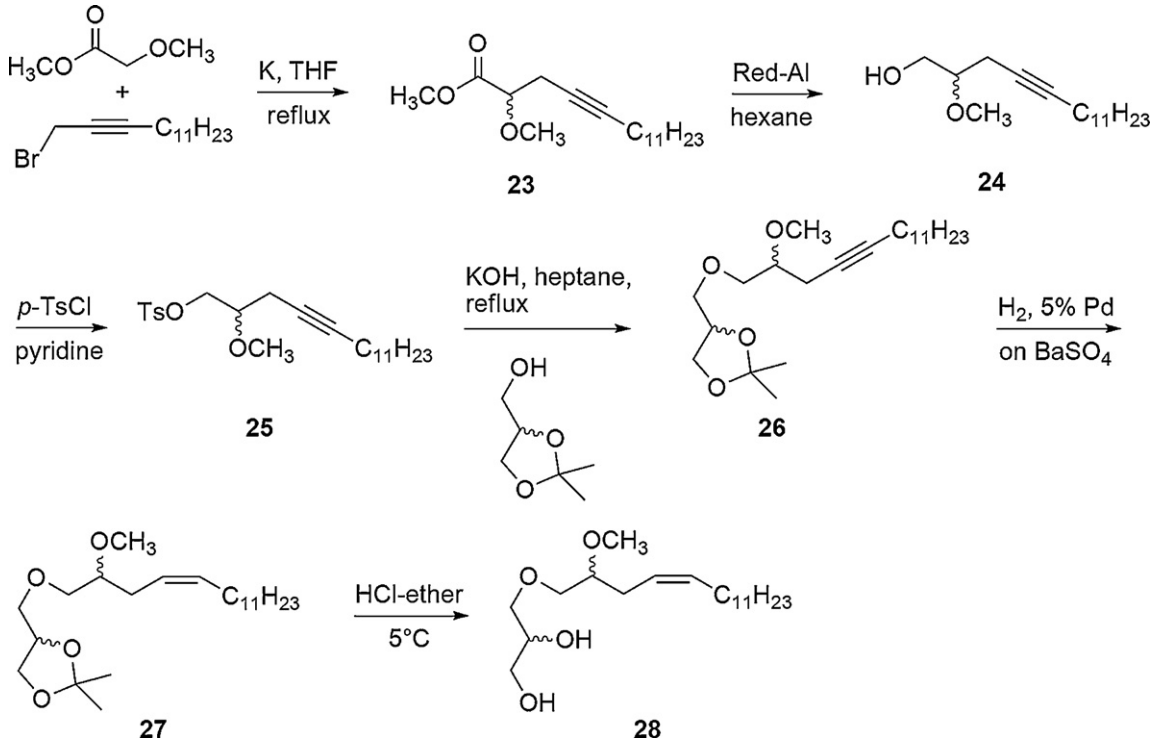


Scheme 5. First synthesis of 1-*O*-(2′-methoxyhexadecyl)glycerol **22** as a mixture of stereoisomers.

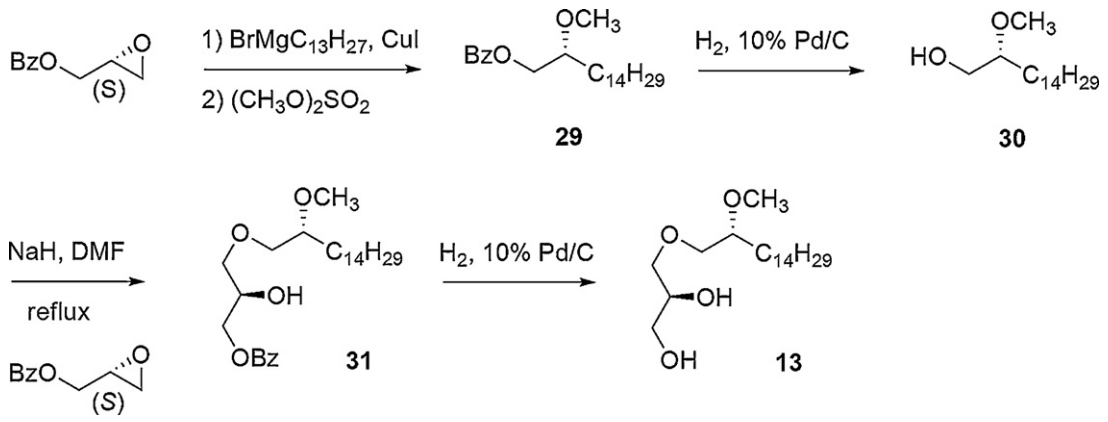
Finally, **33** was hydrolyzed in a dioxane-hydrochloric acid solution, affording **13** in 63% and 24% overall yields.

Magnusson and Haraldsson (2010) have achieved the synthesis of enantiomerically pure (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol **14** (Scheme 9). It was started with the regioselective opening of (*R*)-epichlorohydrin with tridec-1-ynyllithium, obtained by treatment with *n*-butyllithium, in the presence of boron trifluoride according to Yamaguchi and Hirao (1983), affording (*R*)-1-chlorohexadec-4-yn-2-ol **34** in 50% yields and 98% ee (based on ^{1}H NMR analysis (400 MHz) of Mosher ester derivative of **34**).

Subsequent hydrogenation of **34** over Lindlar catalyst, afforded the chloroalkenol **35**, comprising a *cis*-configured double bond in its 4-position, in 96% yields. Then, **35** was reacted with (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol in the presence of ground potassium hydroxide and tetra-*n*-butylammonium bromide, affording the key intermediate (*Z*)-(2′*R*)-1-*O*-(hexadec-4′-en-2′-ol)-2,3-*O*-isopropylidene-*sn*-glycerol **36** in 62% isolated yields.



Scheme 6. First synthesis of (*Z*)-1-*O*-(2′-methoxyhexadec-4′-enyl)glycerol **28** as a mixture of stereoisomers.



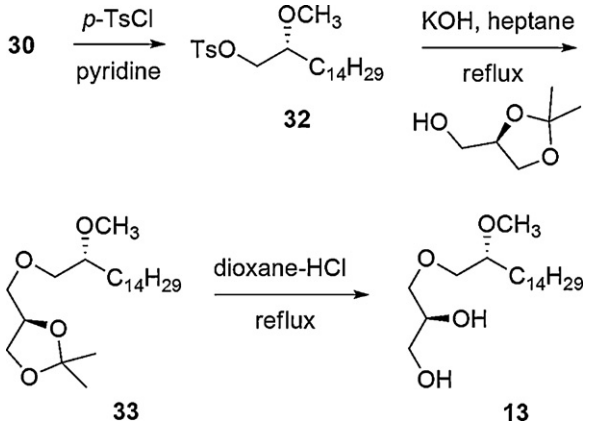
Scheme 7. First synthesis of enantiomerically pure (2′*R*)-1-*O*-(2′-methoxyhexadecyl)-*sn*-glycerol **13**.

Subsequent methylation of the hydroxyl group in **36** with silver oxide and methyl iodide in the presence of molecular sieves in toluene under mild reaction conditions, yielded (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-2,3-*O*-isopropylidene-*sn*-glycerol **37** in 93% yields. Cleavage of the acid labile ketal under mild reaction conditions with Amberlyst 15 in 96% ethanol, furnished the desired final product (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol **14** in 99% yields and 27% overall yields from (*R*)-epichlorohydrin. The right stereochemistry of **14** was confirmed (Magnusson and Haraldsson, 2010) by firstly, comparing the ^{1}H NMR spectrum of **13**, obtained by hydrogenation of **14**, with that of **13** from literature (Stallberg, 1990), to confirm the type of diastereomer, and secondly, by comparison of the optical activities of the afforded **13** and **14** with those found in literature (Stallberg, 1975, 1990), to confirm the type of enantiomer.

3.4. Isolation, identification and structural elucidation

Most of the reports on the isolation of the 2′-methoxylated 1-*O*-alkyl-*sn*-glycerols begin with the alkaline hydrolysis of the non-polar lipid fractions previously extracted from tissue samples by the methods developed by Bligh and Dyer (1959) or Folch et al. (1957). Then, the obtained unsaponifiable matter is chromatographed on silicic acid column and the 2′-methoxyl substituted alkylglycerols, that have a slightly lower Rf value than the unsubstituted alkylglycerols, normally elute accompanied with some unsubstituted alkylglycerols. The mixture product can be further purified by preparative TLC to afford pure 2′-methoxylated alkylglycerols that are subsequently converted into their isopropylidene or dimethoxylated derivatives. On the other hand, the mixture of glyceryl ethers can be first derivatized into their corresponding acetonides and then purified by column chromatography to obtain the acetone-protected 2′-methoxylated alkylglycerols. GLC and GLC-MS analyses of these derivatives have been used for the structural elucidation of the 2′-methoxylated alkylglycerols and for more routine analyses to determine their alkyl composition in different tissue samples (Hallgren et al., 1974a,b, 1971; Hallgren and Stallberg, 1967; Hayashi and Takagi, 1982).

From the mass spectra of the derivatives, the fragmentation patterns indicate that the methoxyl group is located at the 2-position of the alkyl chain. Comparisons of the GLC chromatograms obtained for the isopropylideneglycerol derivatives, before and after their hydrogenation, have been used to demonstrate that unsaturation of the alkyl chains is primarily due to monounsaturation. Indeed, the fragmentation pattern obtained from MS spectra of the 2′-methoxylated alkylglycerol derivatives after treatment with osmium tetroxide and subsequent methylation or trimethylsilylation, have been used to determine the position of the double bonds at the alkyl chain (Hallgren and Stallberg, 1967; Hallgren et al., 1971; Hayashi and Takagi, 1982). Finally, the information obtained from GLC and GLC-MS analyses together with NMR spectroscopy of 2′-methoxylated alkylglycerols from synthetic and natural origin have unambiguously confirmed the final structure of the most prevalent 2′-methoxylated alkylglycerols and their absolute configuration (Hallgren and Stallberg, 1967; Hallgren et al., 1971; Stallberg, 1990).

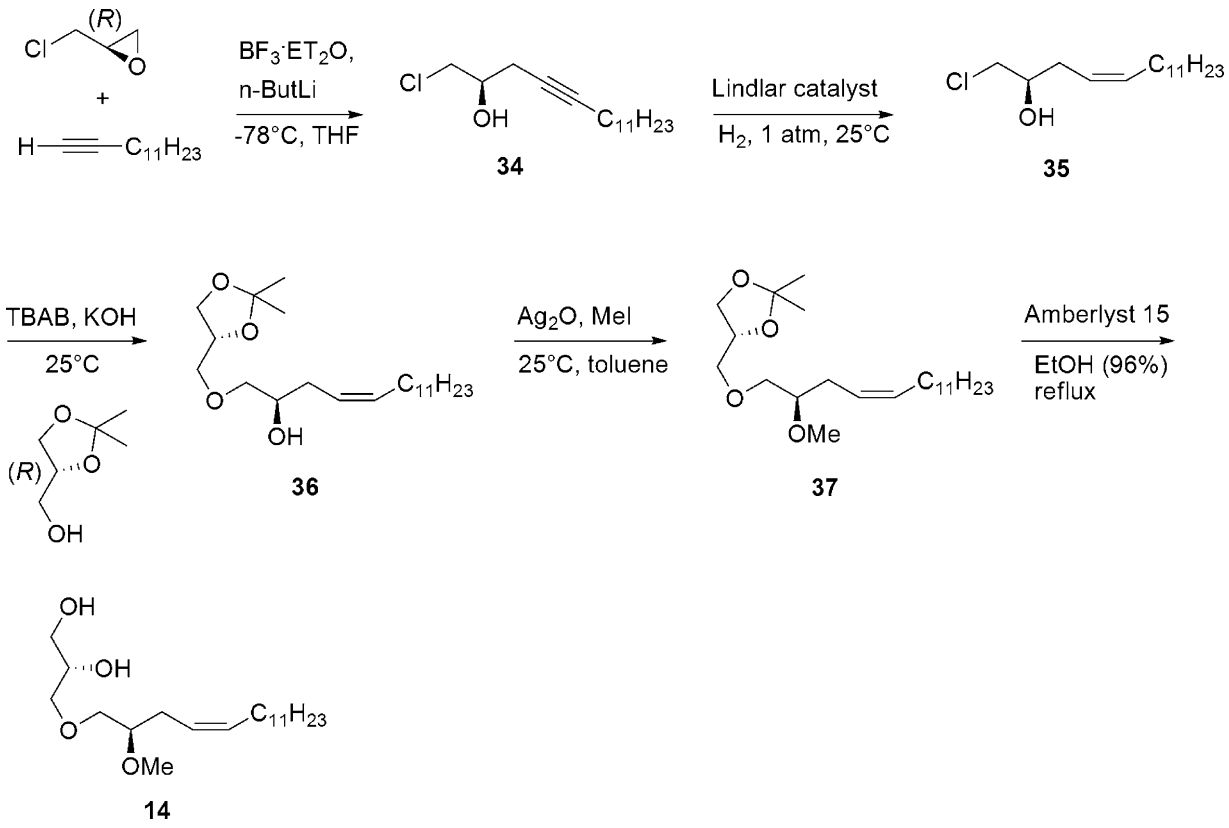


Scheme 8. First synthesis of enantiomerically pure (2′*R*)-1-*O*-(2′-methoxyhexadecyl)-*sn*-glycerol **13**.

3.5. Biological effects

Naturally occurring 2′-methoxylated alkylglycerols isolated from shark liver oil, as well as a synthetic stereoisomeric mixture of 1-*O*-(2′-methoxyhexadecyl)glycerol, have shown antibacterial activity levels similar to those shown by nitrofurantoin against various types of bacteria, including *Corynebacterium Hofmanii*, *Diplococcus pneumonia*, *Staphylococcus pyogenes* A, *Staphylococcus pyogenes* (H. Oxford), *Streptococcus pyogenes* and *Streptococcus viridians* (Boeryd et al., 1971).

2′-Methoxylated alkylglycerols have also exhibited fungistatic effects toward some strains of dermatophytes, *Epidermophyton floccosum*, *Microsporum canis*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. It is notable, that the mixture of 2′-methoxylated alkylglycerols isolated from Greenland shark liver oil at a concentration of 100 μg/ml inhibited dermatophyte growth in the range of 25–60%. On the other hand, the synthetic stereoisomeric



Scheme 9. Synthesis of (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol **14**.

mixture of 1-*O*-(2′-methoxyhexadecyl)-glycerol and (*Z*)-1-*O*-(2′-methoxyhexadec-4′-enyl)glycerol showed only inhibition of 5–15% at the same concentration (Hallgren et al., 1978).

Synthetic 1-*O*-(2′-methoxyhexadecyl)glycerol (as a mixture of stereoisomers) inhibited the human colon cancer cell lines Moser, HT29 and HCT116 to a similar degree with IC_{50} values ranging from 11 to 14 μM and an 80% maximal inhibition at 25 μM concentration *in vitro* (Wang et al., 1999). When the same 2′-methoxyl substituted alkylglycerol was used to treat the human prostate cancer cell lines LnCap and DU145, they were inhibited with IC_{50} values of 93 μM and 97 μM, respectively, higher IC_{50} were cytotoxic. This shows that colon cancer cells are relatively more sensitive to 1-*O*-(2′-methoxyhexadecyl)glycerol than prostate cancer cells (Reynolds et al., 2000). The 2′-methoxylated alkyl phosphocholine derivatives, 1-*O*-(2′-methoxyhexadecyl)-*sn*-glycero-3-phosphocholine and (*Z*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycero-3-phosphocholine, isolated from the marine sponge *Spirastrella abata*, have been assayed for their cytotoxicity against five human tumour cell lines, A549 (human lung cancer), SK-OV-3 (human ovarian cancer), SK-MEL-2 (human skin cancer), XF498 (human CNS cancer) and HCT15 (human colon cancer). Both analogues displayed significant cytotoxicity with ED_{50} (dose that is effective in 50% test subject), ranging from 4.5 to 5.8 μg/ml for the saturated analogue and 3.8 to 6.3 μg/ml for the Δ4 monounsaturated one. It is of interest that the Δ4 methoxylated analogue is slightly more potent (ED_{50} values 3.8 (SK-MEL-2), 4.0 (XF498) and 3.7 (HCT15) μg/ml) against three of the five cancer cells tested, than the saturated analogue (ED_{50} values 5.2 (SK-MEL-2), 4.5 (XF498) and 5.0 (HCT15) μg/ml) (Alam et al., 2001).

A mixture of 2′-methoxyl substituted glyceryl ethers from Greenland shark liver oil has been reported to inhibit metastasis formation from a methylcholanthrene-induced sarcoma (MCG1-SS) to the lymph nodes and lung of CBA mice, when the 2′-methoxylated glyceryl ether mixture was given in a concentration of 0.5% of the diet. The same concentration was not sufficient to achieve tumour growth reduction, but a higher concentration of 2% of the diet significantly inhibited tumour growth, although this is considered an excessive concentration (Boeryd et al., 1971). Likewise, synthetic 1-*O*-(2′-methoxyhexadecyl)glycerol has been reported to inhibit the growth of Melanoma B16 and methylcholanthrene-induced sarcoma (MCG101) in C57BL/6 J mice and of lymphoma LAA in A/Sn mice (Hallgren et al., 1978).

Several *in vivo* studies have demonstrated the immune stimulating properties of 2′-methoxyl substituted alkylglycerols in mice. For instance, oral administration of a mixture of 2′-methoxylated alkylglycerols, isolated from Greenland shark liver oil, and synthetic 1-*O*-(2′-methoxyhexadecyl)glycerol in concentrations of 0.1, 0.25 and 0.5% of the feed, prior to injection of sheep red blood cells (SRBCs), resulted in increased concentration of plaque-forming cells (PFC), indicating stimulation of immune reactivity (Hallgren, 1983). Interestingly, when a mixture of alkylglycerols containing about 3% of the 2′-methoxylated analogues was fed to mice, it was necessary to increase the concentration of the mixture in the diet to 3%, corresponding to about 0.1% of the methoxyl substituted alkylglycerols, to obtain immunostimulation. Therefore, the authors presumed that the methoxylated alkylglycerols may be the only constituents responsible for the immunostimulation activity of the mixture. On the other hand, later *in vivo* studies have demonstrated that unsubstituted alkylglycerols, especially 1-*O*-dodecylglycerol, are potent immunostimulators (Acevedo et al., 2006; Ngwenya and Foster, 1991; Yamamoto and Ngwenya, 1987; Yamamoto et al., 1988). It may be suggested, that perhaps the 2′-methoxylated alkylglycerols may induce a strong immune stimulation similar to that observed for 1-*O*-dodecylglycerol, although a direct comparison of their immunostimulant activity has not been reported, so far.

4. Plasmalogens

Plasmalogens 1-*O*-(alk-1′-enyl)-2-acyl *sn*-glycero-3-phospholipids (**38** and **39** in Fig. 11) belong to the class of ether-phospholipids that possess a *cis* configured double bond at the vinyl-ether function situated at the *sn*-1 position of the glycerol backbone. The major 1-*O*-(alk-1′-enyl) moieties found are palmityl, stearyl and oleyl based. The *sn*-2 postion is usually esterified with a polyunsaturated fatty acid (PUFA), such as DHA or arachidonic acid (ARA). The polar heads commonly found are phosphoethanolamine and phosphocholine, albeit phosphoserine and phosphoinositol are also found (Fig. 11). For convenience, taking into account the variety of polar head groups that plamalogens might have, the prefix *plasmenyl* is commonly used. Thus, a plasmalogen bearing the phosphoethanolamine head is called plasmenyl-ethanolamine and plasmenyl-choline the plasmalogen with a choline head. Likewise, the 1-*O*-alkyl ether-phospholipids bear the prefix *plasmanyl*. Therefore, a 1-*O*-alkyl ether-phospholipid bearing a phosphocholine head is called plasmanylcholine.

Plasmalogens are ubiquitously found in animal cells and in anaerobic microorganisms, but not in plants, with few exceptions (Mangold and Weber, 1987). In mammals, the brain, heart, lymphocytes, spleen, macrophages and polymorphonuclear leukocytes contain the highest amount of plasmenyl-ethanolamine; in contrary the liver has the lowest. Other tissues like, erythrocytes, kidney, lung, testes and skeletal muscle contain moderate amounts (Nagan and Zoeller, 2001).

Plasmalogens constitute about 18% of the phospholipid mass in humans, and plasmenyl-ethanolamine is by far the most abundant ethanolamine phospholipid in mammals (Mangold and Weber, 1987). Their exact biological functions and their mechanisms are still obscure. However, human disorders such as Zellweger syndrome, Rhizomelic chondrodysplasia punctata, Down syndrome, Alzheimer's disease and Nieman-Pick type C characterized by deficiency on plasmalogens, have enlightened some important functions of plasmalogens. Recently, the employment of mice models bearing mutant genes, which elicit deficiency on plasmalogen biosynthesis, have given deeper insight into the main roles of ether-phospholipids, including plasmalogens (Maxwell et al., 2003). According to such studies, several functions have been attributed to plasmalogens, such as to be a source of second messengers, PUFA and lyso-plasmalogens, modulators of membrane dynamics by effect on cell membrane fluidity, mediators of membrane signalling and antioxidant activities. In this manner, plasmalogens have been implicated in signal transduction, myelination, development and preservation of brain tissue and eyes, and development of bones.

4.1. Biological properties

The half-live of plasmenyl-ethanolamine and plasmenyl-choline in brain tissue has been determined, 180 min and 30 min, respectively (Brites et al., 2004). These high turnover rates could be explained by a highly labile vinyl-ether bond, which may act as a scavenger toward oxidative agents in biological systems. For instance, they may be responsible for the protective properties shown to endothelial cells and lipoprotein against oxidative stress (Bittman et al., 2001). Post mortem cortical and cerebellar gray matter phospholipid analysis from Down syndrome patients showed a 35% decrease in plasmenyl-ethanolamine and a 37% decrease in phosphatidylinositol, compared to normal samples (Murphy et al., 2000).

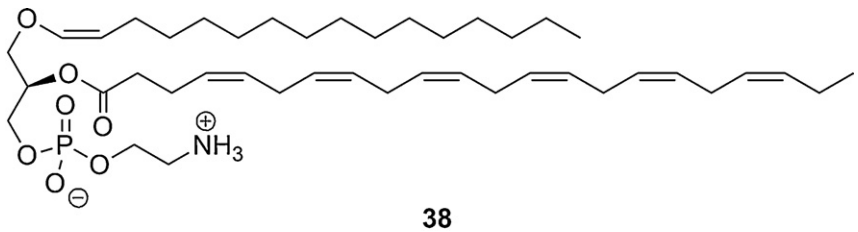
These results suggest plasmalogens to be associated with oxidative stress accompanying Down syndrome as has been reported by studies on mice Down syndrome models (Colton et al., 1990). One study has reported acceleration in the oxidation rate of the vinyl ether bond in 1-*O*-(hexadec-1′-enyl)-2-linoleoyl-3-stearoyl-*sn*-glycerol compared to 1-*O*-(hexadec-1′-enyl)-2,3-distearoyl-*sn*-glycerol. This result clearly showed a deteriorating effect of PUFA on the vinyl ether bond, rather than a protective effect of the vinyl ether bond on PUFA (Foglia et al., 1988). Other supporting oxidation studies on plasmenyl-choline containing ARA noted the peroxidation mechanism of plasmalogens, initiated in the arachidonyl part of plasmalogen (Khaselev and Murphy, 2000). On the other hand, a recent study has shown that the first step of the hypochlorus acid-induced degradation of plasmalogens containing DHA at their *sn*-2 position, is the attack of the vinyl-ether bond (Lessig and Fuchs, 2010). As can be seen, there are contradictory reports on the antioxidant properties of the vinyl ether bond, and thus further oxidation studies on this peculiar vinyl ether bond are needed to settle the controversy.

Studies on Ca^{2+}-induced fusion between phospholipid liposomes demonstrated, that liposomes made of high levels of plasmenyl-ethanolamine comprising ARA at the *sn*-2 position, showed the highest fusion rate. The *cis*-configuration of the double bond in the vinyl-ether chain is suggested to account for the low lamellar liquid crystalline (bilayer phase)-to-hexagonal phase (non-lamellar phase) transition temperature (T_h) of 2-acyl-plasmenyl-ethanolamine (29 °C), compared to 2-acyl-plasmanylethanolamine (46 °C) and phosphatidylethanolamine (53 °C) analogues (Paltauf, 1983b). This particularly low transition temperature of plasmenyl-ethanolamine is likely to facilitate the initial states of membrane fusion (Glaser and Gross, 1994). Furthermore, plasmalogens have been found as major constituents of synaptic vesicle membranes, which may imply their relevant role in chemical synaptic transmission (Gremo et al., 1985).

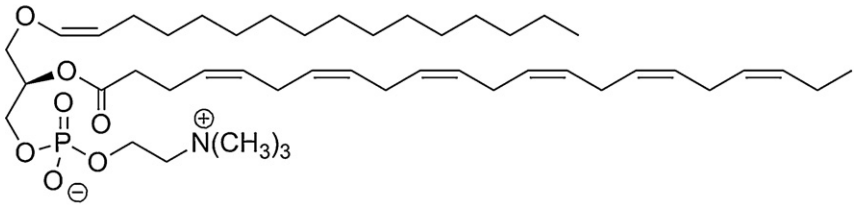
Zellweger syndrome and Rhizomelic chondrodysplasia punctata (RCDP) are genetic diseases characterized by functional impairments in the biosynthetic machinery of plasmalogens, resulting, among other pathological symptoms, in severe depletion of plasmalogens in nervous tissue (Brites et al., 2004). Plasmalogen deficiency is believed to be implicated in neuronal migration defect found in Zellweger patients, therefore claiming them a possible important role in function, distribution and organization of protein membrane, involved in cell-cell junctions, responsible for integral development of nervous tissue. Lipid rafts, specialized cholesterol-enriched membrane domains involved in cellular signalling processes, have been found to contain about 30% higher amount of plasmalogens than plasma membrane (Pike et al., 2002). In addition, their lipid composition has been reported to have a profound influence on signal transduction pathways (Pike et al., 2002).

The implication of plasmalogen depletion in hypomyelination, found in Zellweger syndrome and RCDP patients, together with the fact that lipid rafts in myelin contain high levels of plasmalogens, underline the importance of plasmalogens in function and organization of protein membrane located in lipid rafts, leading to integral signal transduction, which is essential for myelin formation (Gorgas et al., 2006). Plasmalogen deficiency, also observed in other human disorders, such as Alzheimer's disease, may also contribute to degeneration of myelin through their implication in homeostasis of cholesterol, which is an essential constituent of lipid raft, although the mechanism by which plasmalogens act is still not clear (Gorgas et al., 2006).

Plasmalogens store high amounts of DHA and ARA at their *sn*-2 position of the glycerol moiety. Therefore, plasmalogens have been proposed to maintain high pools of PUFA in mammalian cells, such as in brain, heart and spermatozoa. Myelin, the sheath insulation of axons in the central nervous system, is mainly composed of cholesterol, glycolipids and plasmalogens. Plasmenyl-ethanolamine accounts for approximately 80% of the total amount



38



39

Fig. 11. Two prevalent plasmalogens found in human tissue.

of ethanolamine glycerophosphlipids found in brain myelin (Han et al., 2001). Depletion of plasmenyl-ethanolamine and DHA in brain characterizes patients suffering from Zellweger syndrome, leading to serious dysmyelination and multiple neurological abnormalities (Martinez and Mougan, 1999). DHA is an essential fatty acid in nervous tissue and retina, and is therefore implicated in normal brain and retina development, and in cognitive function (Martinez et al., 2010).

Human spermatozoa cells are rich in ether-phospholipids and DHA. Ether-phospholipids (plasmalogens and the 1-*O*-alkyl type analogues), comprising DHA at the *sn*-2 position of the glycerol moiety, have been reported to be one of the major glycerol phopholipid type in spermatozoa (Feki et al., 2004).

Canine myocardial sarcolemmal phospholipids have been reported to contain 40% plasmalogen. Plasmenyl-choline and plasmenyl-ethanolamine constituted 57% and 64% of the PC and PE, respectively. Furthermore, ARA was found esterified at the *sn*-2 position of 75% ethanolamine glycerophospholipid (Gross, 1984).

Plasmalogens containing a highly bioactive fatty acid like DHA or ARA at their *sn*-2 position can be involved in signal transduction by a receptor-mediated hydrolysis at the *sn*-2 position via a phospholipase A_2 selective for ether phospholipids (Gross et al., 1993). Then, the PUFAs can act as intracellular mediators by themselves and, for example, affect gene expression (Terano et al., 1999), or they can serve as precursors for prostaglandin biosynthesis (Morrow and Roberts, 2001; Youdim et al., 2000). Furthermore, lyso-plasmalogens generated as a response of myocardial ischemia have been reported to elicit several electrophysiological alterations, contributing to arrhythmogenesis in ischemic heart (McHowat et al., 1998).

5. Conclusions

Ether lipids of the 1-*O*-alkyl-*sn*-glycerol type are ubiquitously found in nature, although primarily in the animal kingdom. In general, they are more prevalent in the phospholipids, where they are found as alkyl acyl phosphatides, than in the neutral lipids, where they exist as 1-*O*-alkyl-2,3-diacyl-*sn*-glycerols. In contrast, the liver oils of some deep-sea shark species of the Squaliformes order are highly enriched with the diacylated form of the 1-*O*-alkyl-*sn*-glycerols. Such shark species have indeed high DAGE to TAG ratios and are a potential source of high quality shark liver oil for industry.

The saturated and monounsaturated *O*-alkyl chains of 16 and 18 carbon atoms account for about 80% of the 1-*O*-alkyl composition of DAGEs. Recent studies have revealed that, at least in mammals, the fatty acyl specificity of two acyl-CoA reductase isoenzymes, FAR1 and FAR2, might be responsible for the relatively narrow spectra of 1-*O*-alkyl chains found in the 1-*O*-alkyl-*sn*-glycerols. Furthermore, different tissue distributions of these reductases may explain the varying concentrations of the major alkylglycerols in different animal tissues.

The most efficient synthetic approaches toward enantiomerically pure unsubstituted 1-*O*-alkyl-*sn*-glycerols are based on the Williamson ether synthesis between the chiral C_3-synthon 2,3-*O*-isopropylidene-*sn*-glycerol and the corresponding alkyl sulfonates or halides under alkaline conditions, followed by mild acid catalyzed hydrolysis of the isopropylidene moiety.

Contradictory results related to higher concentrations of 1-*O*-alkyl and 1-*O*-(alk-1′-enyl) glycerol based ether lipids in mammalian cancer cells, compared to normal healthy cells, have obstructed the application of such concentration differences as a valid parameter for medical diagnosis of cancer. Nevertheless, it may be concluded that tumour cells usually exhibit changes in their ether lipid levels, besides variations in their 1-*O*-alkyl, 1-*O*-(alk-1′-enyl) and fatty acyl compositions.

High DAGE levels encountered in the liver oil of some cartilaginous fish species and, more recently, in the pterpod *Clione limacina*, are thought to play an important role in their maintenance of buoyancy. This is based on the higher uplift provided by DAGE molecules, due to their lower density, compared to TAG molecules.

In mammals, dietary 1-*O*-alkyl-*sn*-glycerols and their antipodes, 3-*O*-alkyl-*sn*-glycerols, are completely absorbed in the gut, but only those of the natural *sn*-1 configuration are incorporated into DAGEs, neutral plasmalogens, and their phosphoether lipid analogues, plasmanyl- and plasmenyl-phospholipids. A recent report has shown increased levels of ether-linked glycerolipids in the tissues after oral supplementation of 1-*O*-alkyl-*sn*-glycerols in mammals.

1-*O*-alkyl-*sn*-glycerols are bioactive compounds with high therapeutic potential. Among the beneficial properties that have been attributed to them are antineoplastic, antibacterial and antifungal activities. 1-*O*-alkyl-*sn*-glycerols may exert their anti-neoplastic effects by two different routes, firstly, via stimulation of the immune system, and secondly, by hindering vascularisation of tumour cells, which in turn might be mediated by 1-*O*-alkyl-*sn*-glycerol promoted inhibition of certain PKC isoforms, presumably involved in tumour growth and metastasis.

Recently, plasmalogens and plasmanyl-phospholipids have been found to be one of the major glycerol based phospholipids in human spermatozoa. This high prevalence of 1-*O*-alkyl based phospholipids implies their relevant function in fertility and reproduction. In this regard, 1-*O*-alkyl-*sn*-glycerols increased the fertility of spermatozoa in artificial inseminations. A possible mechanism of action is thought to involve concomitant increase in production of lyso-PAF after incubation with naturally occurring 1-*O*-alkyl-*sn*-glycerols.

In the last decade, some efforts have been spent to exploit the amphiphilic properties of 1-*O*-alkyl-*sn*-glycerols as drug carriers. For instance, short-chain 1-*O*-alkylglycerols have been shown to facilitate the delivery of antibiotics and antineoplastic agents into the brain by increasing the permeability of the blood brain barrier. A design prodrug has been prepared by conjugation of (*S*)-batyl alcohol with a highly polar drug in the aiming to increase the drug's bioavailability.

The (2′*R*)-1-*O*-(2′-methoxyalkyl)-*sn*-glycerols are a special category of 1-*O*-alkyl-*sn*-glycerols, whose general structural feature is a methoxyl group attached to the 2-position of the 1-*O*-alkyl moiety. In nature, they occur as minor components of the glyceryl ether mixtures. The 2′-methoxylated alkylglycerols are relatively prevalent among the glyceryl ethers in animals of marine origin. In land mammals, in contrast, they only occur in trace quantities. Like their unsubstituted congeners, the 2′-methoxylated alkylglycerols are usually more prevalent in the phospholipids than in the neutral lipids of marine animals.

In general, the long alkyl chains of 1-*O*-(2′-methoxyalkyl)-*sn*-glycerols range from C14 to C22, and the principal 2′-methoxylated 1-*O*-alkyl chains are hexadec-4′-enyl, hexadecyl and octadec-4′-enyl, although in some cases the octadecyl chain can be found in moderate quantities. The hexadec-4′-enyl methoxylated alkylglycerol is clearly the most abundant methoxylated alkylglycerol found in the liver oil of cartilaginous fish. The monounsaturated chains are characterized by comprising a $\Delta 4$ *Z*-configured double bond.

An unprecedented biosynthesis of the 2′-methoxylated alkylglycerols has been proposed based on, firstly, a possible convergent biosynthetic origin with the α-hydroxylated fatty acids, and secondly, the existence of 2′-hydroxylated alkylglycerols found in the unsaponifiable fraction of shark liver oil.

The major saturated methoxyl substituted alkylglycerol, (2′*R*)-1-*O*-(2′-methoxyhexadecyl)-*sn*-glycerol has been synthesized enantiomerically pure by two closely related approaches, using (*S*)-1-benzyloxy-2,3-epoxypropane and (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol. The synthesis of enantiopure (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol, the most prevalent 2′-methoxylated type alkylglycerol present in cartilaginous fish, has been achieved by a highly convergent five step process, taking place in 27% overall yield. Its key step was an ether bond formation between the chiral synthon (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol and (*Z*)-(*R*)-1-chlorohexadec-4-en-2-ol, employing ground potassium hydroxide and tetra-*n*-butylammonium bromide as a phase-transfer catalyst, under solvent free condition. This is the first report on the synthesis of this enantiopure compound as far as we know. It is anticipated that this facile and efficient synthetic approach will prompt scientists to further investigations on the biological properties of this intriguing compound.

Studies have demonstrated the antibacterial, antifungal and immune stimulant properties of the methoxyl substituted alkylglycerols. Furthermore, their anti-tumour activities, including inhibition of tumour growth and metastasis formation, have been assessed.

Plasmalogens are ether-phospholipids of the (*Z*)-1-*O*-(alk-1′-enyl) type, widely found in animal cells and in anaerobic microorganisms. Their principal role in biological systems have for a long time been unknown, however, researches on patients suffering from diseases characterized by impairments in the biosynthetic machinery of plasmalogens, such as Zellweger syndrome and RCDP, have enlightened some of their relevant functions. The structural characteristic vinyl-ether function of plasmalogens is believed to be involved in their role as scavengers of oxidation agents and to strongly influence the cell membrane dynamic properties. The high prevalence of PUFAs in the *sn*-2 position of the glycerol backbone of plasmalogens implies a close relation between the functions of these two compounds. Scientists have just begun the understanding of plasmalogens and PUFAs and, hopefully, further studies will provide us with a deeper knowledge on the functional interactions of these two highly bioactive compounds.

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