



Review

New advances in the chemistry of methoxylated lipids

N.M. Carballeira*

Department of Chemistry, University of Puerto Rico, PO Box 23346, San Juan, Puerto Rico 00931-3346

Abstract

Methoxylated lipids have been reviewed emphasizing the alkylglycerol ethers and fatty acids bearing the methoxy group in the alkyl chain. The literature on methoxylated lipids and their derivatives has been divided into four main groups, namely 2-methoxylated alkyl glycerols, ω -methoxylated fatty acids, mid-chain methoxylated fatty acids, and α -methoxylated fatty acids. The natural occurrence, biological activity, and synthesis of this interesting group of lipids are discussed. Most of these compounds have been isolated from either bacterial or marine sources, but others are mainly of synthetic origin. Among the interesting biological activities displayed by these compounds the most important are antibacterial, antifungal, antitumor, and antiviral. © 2002 Elsevier Science Ltd. All rights reserved.

Contents

1. Introduction	438
2. 1- <i>O</i> -(2-Methoxyalkyl)glycerols and derivatives	439
2.1. Naturally occurring	439
2.2. Biological studies	440
2.3. Synthetic studies	441
3. ω -Methoxylated fatty acids and derivatives	442
3.1. Naturally occurring	442
3.2. Biological studies	443
3.3. Synthetic studies	444

* Tel.: +1-787-764-0000x4791; fax: +1-787-756-8242.

E-mail address: ncarbball@upracd.upr.clu.edu

4.	Mid-chain methoxylated fatty acids and derivatives	444
4.1.	Naturally occurring	444
4.1.1.	Bacterial origin	444
4.1.2.	Marine origin	445
4.2.	Biological studies	446
4.3.	Synthetic studies	447
5.	α -Methoxylated fatty acids and derivatives	448
5.1.	Naturally occurring	448
5.2.	Biological studies	451
5.3.	Synthetic studies	451
6.	Summary	454
	Acknowledgements	455
	References	455

Nomenclature

AZT	Azidothymidine
<i>n</i> -BuLi	<i>n</i> -Butyllithium
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
FLT	Fluorodeoxythymidine
HBV	Human liver hepatitis B
HIV-1	Human immunodeficiency virus I
MIC	Minimum inhibitory concentration
MoMLV	Moloney murine leukemia virus
NMR	Nuclear magnetic resonance
NMT	<i>N</i> -Myristoyltransferase
PAF	Platelet activating factor
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
SAM	S-Adenosyl-L-methionine
THF	Tetrahydrofuran
TMS-CN	Trimethylsilyl cyanide

1. Introduction

Methoxylated lipids have been known for almost half a century [1]. Despite their occasional occurrence in nature much research has been carried out with this intriguing group of lipids. Many display important biological activities, such as cytotoxicity, while others have only been synthesized for characterization purposes or even as artifacts in the transesterification of lipids. It

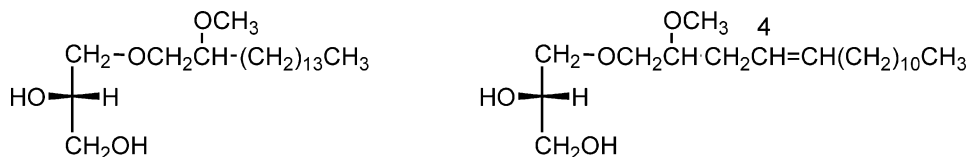


Fig. 1. The structures of 1-*O*-(2-methoxyhexadecyl)glycerol and 1-*O*-(2-methoxy-4-hexadecenyl)glycerol.

is the aim of this contribution to summarize the recent literature dealing with methoxylated lipids and to put it in perspective with previous work envisioning that a more comprehensive picture of the field emerges. In doing so we hope to have identified gaps in the literature that could lead to new research endeavors.

In this work we have concentrated on lipids bearing the methoxy group in the alkyl chain. This led us to divide the literature on methoxylated lipids and their derivatives into four main groups, namely the 2-methoxylated alkyl glycerols, ω -methoxylated fatty acids, mid-chain methoxylated fatty acids, and the α -methoxylated fatty acids. We have not included in this review the ether-linked lipid 1-*O*-octadecyl-2-*O*-methyl-rac-glycero-3-phosphocholine (ET-18-OCH₃) and its derivatives, which have received considerable literature attention in the past for its cytotoxicity toward several types of tumor cells. To the best of our knowledge the methoxylated lipids in this contribution have not been collectively reviewed in the past.

2. 1-*O*-(2-Methoxyalkyl)glycerols and derivatives

2.1. Naturally occurring

One of the earliest methoxylated lipids discovered were the 2-methoxyalkyl glycerol ethers first isolated by Hallgren et al. from Greenland shark liver oil, where they constituted about 4% of the total glycerol ethers [1]. These first glycerol ethers were characterized as 1-*O*-(2-methoxyhexadecyl)glycerol, 1-*O*-(2-methoxy-4-hexadecenyl)glycerol, and 1-*O*-(2-methoxy-4-octadecenyl)glycerol (Fig. 1). These alkylglycerols were characterized by mass spectrometry of the corresponding 2,3-*O*-isopropylidene derivatives and the structure of 1-*O*-(2-methoxyhexadecyl)glycerol was confirmed by total synthesis [1]. More recently the same 1-*O*-(2-methoxyhexadecyl)glycerol and 1-*O*-(2-methoxy-4-hexadecenyl)glycerol (Fig. 1) first discovered by Hallgren were again identified by Pietra et al. in the brachiopod *Gryphus vitreus* collected in the Tuscan archipelago [2].

Further isolation efforts by the Hallgren group resulted in the identification, in trace amounts, of a complete series of 1-*O*-(2-methoxyalkyl)glycerols in the neutral lipids and phospholipids of human colostrum, human milk, cow's milk, sheep's milk, human red bone marrow, red cells, blood plasma, and an uterine carcinoma [3]. The principal components were again the 2-methoxy-substituted hexadecyl-, hexadecenyl-, and octadecenyl glycerol ethers. A characteristic feature of these methoxy substituted glycerol ethers was the predominance of ethers with 16 carbon atoms in the long alkyl chain. The rather unusual polyunsaturated 1-*O*-(2-methoxydocosahexaenyl) glycerol was also identified in red blood cells [3].

1-*O*-(2-Methoxyalkyl)glycerols were also initially identified in the neutral lipids and phospholipids of a series of marine sources such as herring fillets, Baltic herring fillets, macherel fillets, marine

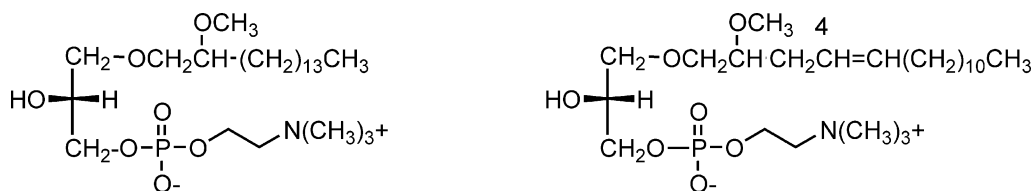


Fig. 2. The structures of 1-*O*-(2-methoxyhexadecyl)-*sn*-glycero-3-phosphocholine and 1-*O*-(2-methoxy-4*Z*-hexadecenyl)-*sn*-glycero-3-phosphocholine.

crayfish, fresh-water crayfish, shrimps and sea mussels [4]. The long-chain components of the methoxy-substituted glycerol ethers ranged in length between 14 and 22 carbon atoms, but again the C₁₆, C_{16:1}, and C_{18:1} chain-lengths predominated in these lipids.

More recently, a novel series of lysophosphatidylcholines were isolated from the marine sponge *Spirastrella abata*, collected in Cheju Island, Korea [5]. In particular, two of these lysophosphatidylcholines were methoxylated lipids, namely the phosphocholines 1-*O*-(2-methoxyhexadecyl)-*sn*-glycero-3-phosphocholine and 1-*O*-(2-methoxy-4*Z*-hexadecenyl)-*sn*-glycero-3-phosphocholine (Fig. 2). These compounds were fully characterized by modern nuclear magnetic resonance (NMR) techniques. Interestingly enough, these lyso-PAF (platelet activating factor) congeners are the corresponding phospholipids of the methoxylated alkylglycerols first isolated by Hallgren [1].

2.2. Biological studies

Since their isolation in the late sixties the 2-methoxyalkyl glycerol ethers stimulated a lot of research for the variety of biological activity that they displayed, such as antibacterial, antifungal, antitumor, and immune-stimulant activity [6,7]. Earlier studies established that these compounds inhibited metastasis formation of the methylcholanthrene-induced sarcoma MCGI-SS in syngeneic CBA mice [6]. The 2-methoxyalkyl glycerol ethers also displayed an ample spectrum of antibiotic activity against several types of bacteria, among them *Corynebacterium hofmannii*, *Diplococcus pneumoniae*, *Staphylococcus pyogenes* (A) and *Staphylococcus pyogenes* (H), *Streptococcus pyogenes* and *Streptococcus viridans* [6]. In addition, the methoxylated alkylglycerols inhibited the growth of HeLa cells in vitro and were later shown to stimulate immune reactivity [8].

Among the different ether lipids reported, the 1-*O*-(2-methoxyhexadecyl)glycerol has received the most attention in recent years. Earlier work with a series of tumor-host systems revealed that 1-*O*-(2-methoxyhexadecyl)glycerol had the best inhibitory effect on Melanoma B16, Lewis lung tumor, MCA-sarcoma MCG101 and the lymphomas LAA and P1534 [9]. New findings have demonstrated that this naturally occurring lipid also inhibits cellular proliferation, anchorage-independent growth, and cellular invasion in the human prostate cancer LnCap and DU145 cells. In particular, 1-*O*-(2-methoxyhexadecyl)glycerol inhibits cell growth with an IC₅₀ of 93 μM for LnCap and 97 μM for DU145. The authors pointed out the importance of this ether lipid in the chemoprevention of malignant diseases, a topic that surely warrants further investigation [10].

The 1-*O*-(2-methoxyhexadecyl)glycerol has also been tested against three human colon cancer cell lines [11]. These were the moderately differentiated and growth factor-responsive Moser, the

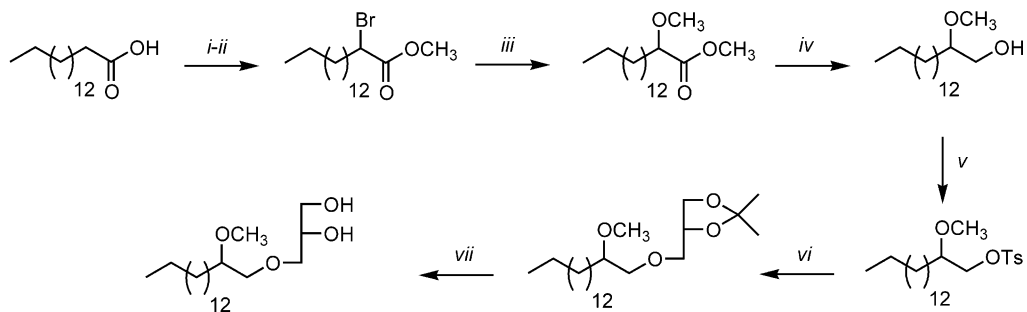


Fig. 3. The first synthesis of racemic 1-*O*-(2-methoxyhexadecyl)glycerol [1]. (i) $\text{SOCl}_2/\text{Br}_2$; (ii) MeOH; (iii) NaOMe/MeOH; (iv) $\text{LiAlH}_4/\text{Et}_2\text{O}$; (v) *p*-TsCl/pyridine; (vi) 1,2-isopropylidene glycerol/NaH; (vii) 0.5 N HCl/100 °C 3 h.

growth factor-unresponsive and malignant HT29, and the poorly differentiated and growth factor-unresponsive HCT116. The 1-*O*-(2-methoxyhexadecyl)glycerol inhibited the growth of these cell lines with IC_{50} 's between 11 and 14 μM . It was also concluded that 1-*O*-(2-methoxyhexadecyl)glycerol promoted a more benign or differentiated phenotype in these colon cancer cells [11].

The phospholipids 1-*O*-(2-methoxyhexadecyl)-*sn*-glycero-3-phosphocholine and 1-*O*-(2-methoxy-4*Z*-hexadecenyl)-*sn*-glycero-3-phosphocholine, isolated from *S. abata*, are significantly cytotoxic against a series of human solid tumor cells lines with ED_{50} 's between 3.7 and 6.3 $\mu\text{g}/\text{ml}$ [5]. Among the cell lines tested were the A549 human lung cancer, SK-OV-3 human ovarian cancer, SK-MEL-2 human skin cancer, XF498 human CNS cancer and the HCT15 human colon cancer. The monounsaturated lysophosphatidylcholine showed a slightly better cytotoxicity against the SK-MEL-2 and HCT15 cell lines, i.e., ED_{50} 's of 3.7–3.8 vs ED_{50} 's of 5.0–5.2. In this bioassay the 1-*O*-(4*Z*-hexadecenyl)-*sn*-glycero-3-phosphocholine was inactive. Therefore, both the methoxylated alkylglycerols and the methoxylated lysophosphatidylcholines display antitumor activity.

2.3. Synthetic studies

Several syntheses for 1-*O*-(2-methoxyhexadecyl)glycerol have been reported [12–16]. The first syntheses were achieved by Hallgren and Ställberg [1] and by Rozin et al. [12,13], but the latter also synthesized the corresponding 1-*O*-(2-methoxyalkyl)-2-acyl-*sn*-glycero-3-phosphoethanolamines and phosphocholines. Naturally occurring alkylglycerols belong to the 1-*O*-alkyl-*sn*-glycerol series (2*S*-configuration), but the absolute configuration at the carbon bearing the methoxy functionality was initially unknown. Therefore, the first syntheses afforded racemic products (Fig. 3). A typical synthesis started with hexadecanoic acid, which was α -brominated with thionyl chloride and bromine and esterified to methyl 2-bromohexadecanoate with the addition of methanol in an 81% yield (Fig. 3). Subsequent nucleophilic displacement of the α -bromine with sodium methoxide in methanol afforded methyl 2-methoxyhexadecanoate in a 95% yield. Reduction of the methyl ester with lithium aluminum hydride (LiAlH_4) and tosylation with *p*-toluenesulfonyl chloride in pyridine yielded the expected 2-methoxyhexadecyl *p*-toluenesulfonate. The methoxyhexadecyl *p*-toluenesulfonate was then coupled with 1,2-isopropylidene glycerol (45% yield) affording 2,3-*O*-isopropylidene-1-*O*-(2-methoxyhexadecyl)glycerol, which after deprotection with 0.5 N HCl yielded the desired 1-*O*-(2-methoxyhexadecyl)glycerol in an overall 21% yield from palmitic acid.

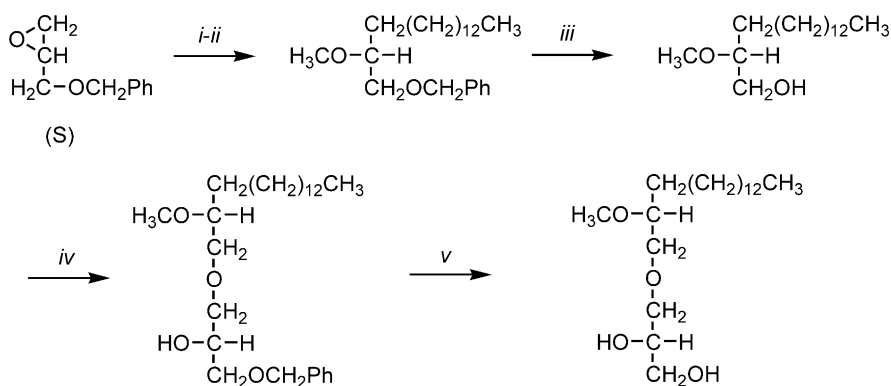


Fig. 4. Synthesis of (2*R*)-1-*O*-(2-methylhexadecyl)-*sn*-glycerol [17]. (i) CH₃(CH₂)₁₂MgBr, CuI/THF; (ii) (CH₃O)₂SO₂; (iii) H₂/THF; (iv) (*S*)-1-benzyloxy-2,3-epoxypropane, NaH/DMF; (v) H₂, 10% Pd/C, THF.

A more recent synthesis of the four stereoisomers of 1-*O*-(2-methoxyhexadecyl)glycerol established the absolute *R* stereochemistry for the carbon bearing the methoxy group in the alkyl chain by comparing the ¹H NMR spectra of the synthetic diastereomers with the natural compound [17]. The synthesis of the natural methoxyalkyl glycerol started with (*S*)-1-benzyloxy-2,3-epoxypropane which was reacted with the Grignard reagent of 1-bromotridecane in the presence of copper iodide and anhydrous tetrahydrofuran (THF) followed by the addition of dimethyl sulfate which afforded (*R*)-1-benzyloxy-2-methoxyhexadecane in a 69% yield (Fig. 4). The latter product was hydrogenated (H₂) over 10% Pd/C in THF as solvent so as to remove the benzyl group and this yielded in a 91% yield (*R*)-2-methoxyhexadecan-1-ol. Reaction of the latter alcohol with (*S*)-1-benzyloxy-2,3-epoxypropane in the presence of a suspension of sodium hydride in anhydrous dimethylformamide (DMF) afforded (2*R*)-1-*O*-(2-methoxyhexadecyl)-3-*O*-benzyl-*sn*-glycerol in a 43% yield. Final deprotection with hydrogen in 10% Pd/C and THF as solvent yielded the naturally occurring (2*R*)-1-*O*-(2-methoxyhexadecyl)-*sn*-glycerol.

3. ω-Methoxylated fatty acids and derivatives

3.1. Naturally occurring

There are almost no examples of naturally occurring ω-methoxylated fatty acids since most of these lipids, such as the 12-methoxydodecanoic acid, are synthetic compounds. Probably the only report in the literature pertains to a ω-methoxylated fatty acid identified in the lipopolysaccharides from the acidophile *Thiobacillus ferrooxidans* grown on iron, sulfur or glucose as energy sources [18]. Analysis of the methyl ester derivatives of the fatty acids from *T. ferrooxidans* by gas chromatography–mass spectrometry revealed the presence of a methoxy methyl ester tentatively identified as CH₃OC₁₀H₁₀CO₂CH₃ as the major component from each lipopolysaccharide [18]. Limitations of cell yields prevented the researchers from obtaining enough material to actually isolate the unusual methoxylated fatty acid. This means that more work was needed to determine the structure of this unorthodox fatty acid as well as its functional role in these lipopolysaccharides.

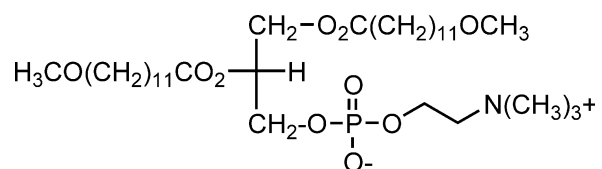


Fig. 5. Symmetrical phosphatidylcholine containing the 12-methoxydodecanoic acid.

3.2. Biological studies

This is probably the most studied area for this type of lipids. Initial work by Gordon et al. demonstrated that the 12-methoxydodecanoic acid, a heteroatom-containing analog of myristic acid with hydrophobicities equivalent to dodecanoic or decanoic acids, inhibits both the human immunodeficiency virus I (HIV-I) and the Moloney murine leukemia virus (MoMLV) [19]. The methoxylated acid was probably incorporated into specific *N*-myristoyl proteins, i.e., oncoproteins and viral proteins critical for assembly/replication, without associated cellular toxicity. The enzyme myristoyl-CoA: protein *N*-myristoyltransferase (NMT; EC 2.3.1.97) seems to recognize chain length rather than hydrophobicity and catalyzes the co-translational transfer of myristic acid from myristoyl-CoA to the amino-terminal glycine residue of a number of proteins [19]. More recently, a series of myristic acid analogs, designed as alternative substrates for *N*-myristoyltransferase, were synthesized and from this series the 12-methoxydodecanoic acid also exhibited in vitro activity ($\text{EC}_{50} = 20\text{--}30 \mu\text{M}$) against human liver hepatitis B virus (HBV) producing 2.2.15 cell lines [20]. Myristoylation by unnatural myristic acid analogues seems to block the synthesis of the PreS1 protein in the membrane envelope of HBV [21,22].

Despite the fact that the 12-methoxydodecanoic acid inhibits the replication of the human immunodeficiency virus (HIV) in infected cells by acting as an alternate substrate in the viral protein myristoylation event, intact phospholipids seem to exert a stronger effect. For example, a symmetric *L*- α -phosphatidylethanolamine containing 12-methoxydodecanoic acid at both the *sn*-1 and *sn*-2 positions was 40-fold more antiviral than the parent methoxylated fatty acid [23]. Moreover, it was also shown that a phosphatidylcholine analog (Fig. 5) has a better antiviral selectivity, is 7-fold more potent, and is synergistic with azidothymidine (AZT). The synergism with AZT can be ascribed to two different mechanisms since AZT is a reverse transcriptase inhibitor and the methoxylated phospholipids inhibit endogenous myristoylation of the HIV proteins. Both the *D*- and *L*-phospholipid stereoisomers had equal antiviral activity and this suggested that the antiviral activity does not depend on phospholipase A_2 enzymatically liberating the methoxylated fatty acids [23].

The membrane properties of the antiviral 1,2-bis(12-methoxydodecanoyl)-*sn*-3-phosphocholine (Fig. 5) have been studied by Pidgeon et al. [23]. The main finding was that micelles formed by sonication with this phospholipid are not thermodynamically stable because they fuse into larger unilamellar vesicles that are stable to further changes in temperature [24]. It was also observed that these methoxylated phosphocholines do not contain an aqueous core that is the characteristic morphology of conventional lipid vesicles formed by sonication.

ω -Methoxylated fatty acid prodrugs have been synthesized with unexpected specificity [25]. Among these we should mention the 5'-*O*-(12-methoxydodecanoyl) esters of 3'-fluoro-2',3'-dideoxythymidine (FLT) and 3'-azido-2',3'-dideoxythymidine (AZT) (Fig. 6). AZT is relatively ineffective

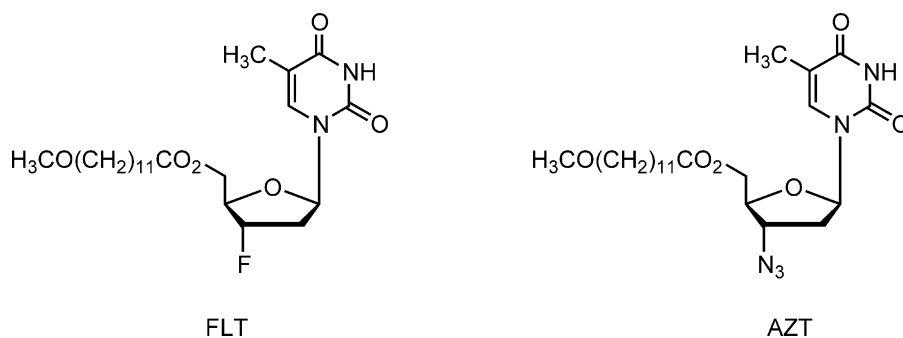


Fig. 6. 5'-*O*-(12-methoxydodecanoyl) ester derivatives of 3'-fluoro-2',3'-dideoxythymidine (FLT) and 3'-azido-2',3'-dideoxythymidine (AZT).

against hepatitis B virus (HBV) replication in 2.2.15 cells, even at high concentrations. However, the 5'-*O*-(12-methoxydodecanoyl) ester of AZT showed modest activity ($EC_{50} = 2.7 \pm 0.3 \mu M$) and low toxicity ($CC_{50} = 727 \pm 19 \mu M$) in 2.2.15 cell culture (HBV virion extracellular DNA). The FLT derivative was also active with an $EC_{50} = 2.8 \pm 0.3 \mu M$ and a CC_{50} of $186 \pm 20 \mu M$. These ester prodrugs were designed so as to have two anti-HBV agents (nucleoside and myristic acid analogue) in the same molecule. Either AZT or FLT is expected to inhibit reverse transcriptase and the myristic acid analogue to inhibit *N*-myristoyltransferase. This means two different mechanisms of action for greater therapeutic efficacy. In addition, the non-polar myristoyl analogue was expected to increase the lipophilicity of the molecule, which could translate into a larger dose of the compound being delivered to the liver. Despite these findings, more research is needed to fully understand the mechanism of anti-HBV activity exhibited by these modified myristoyl compounds [25].

3.3. Synthetic studies

The key synthesis in this type of fatty acids, in particular that of 12-methoxydodecanoic acid, was accomplished by treating the corresponding ω -halocarboxylic acid with sodium methoxide as shown below [26].



4. Mid-chain methoxylated fatty acids and derivatives

4.1. Naturally occurring

4.1.1. Bacterial origin

There have been several reports of mid-chain methoxylated fatty acids in the literature. For example, from the acid-producing bacterium *Thiobacillus* four unusual mid-chain methoxylated fatty acids were characterized. These fatty acids were identified as 10-methoxyoctadecanoic acid,

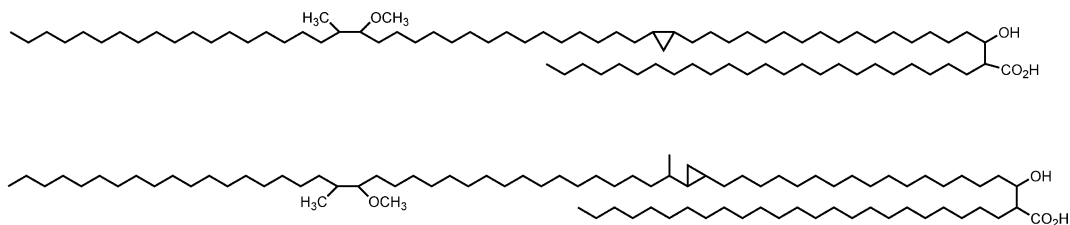


Fig. 7. Representative structures of the methoxymycolic acids.

11-methoxyoctadecanoic acid, 12-methoxyeicosanoic acid, and the 13-methoxyeicosanoic acid [27]. However, there are several reports where these methoxylated fatty acid derivatives are simply the products of chemical alterations of common cyclopropane-containing and unsaturated fatty acids occurring during various acid-catalyzed transesterification treatments aimed at producing the methyl ester derivative [28]. Therefore, the finding of unusual methoxylated fatty acids should be carefully confirmed by either deuterated reagents or simply changing the solvent to ethanol [28,29].

Some recent findings reported the fatty acids 11-methoxyheptadecanoic acid and 11-methoxynonadecanoic acid, after acid methanolysis, in the cholesterol esters and triglycerides of the bacillus *Helicobacter pylori* [30]. It was postulated that these methoxylated lipids, in particular the cholesterol esters may contribute to the acid-resistant properties of *H. pylori*, which is normally encountered in the stomach of humans and this *bacillus* may be involved in the development of stomach cancer [30].

Probably one of the most intriguing fatty acid structures are those of the methoxylated mycolic acids such as those shown in Fig. 7. The methoxylated mycolic acids are produced by some mycobacteria, such as *Mycobacterium tuberculosis*, bacteria that are directly responsible of more human casualties than any other infectious agent [31]. The mycolic acids are long-chain hydroxy fatty acids that range in length between 60 and 80 carbons and contain several functional groups, which in some cases it can be a methoxy group [32]. These methoxylated mycolic acids have been isolated from *M. tuberculosis*, *M. bovis* BCG strain Moreau, *M. microti*, *M. marinum*, *M. ulcerans*, *M. asiaticum*, *M. gastri*, *M. gordonae*, *M. kansasii*, *M. szulgai*, and *M. africanum* [33–38]. In a recent paper the gene MMAS-3, responsible for the biosynthesis of the methoxy group in these methoxylated mycolic acids, was cloned and sequenced [31]. The MMAS-3 enzyme appears to operate on the distant hydroxy group and *O*-methylates using *S*-adenosyl-*L*-methionine (SAM) [31]. This finding seems to have established that in the methoxylated mycolic acids the methoxy functionality arises from the corresponding hydroxy acid via SAM methylation.

4.1.2. Marine origin

Four unprecedented mid-chain methoxylated fatty acids were identified in the red alga *Schizymenia dubyi* collected in Sicily, Italy [39]. By means of gas chromatography–mass spectrometry analyses the methoxylated fatty acids were characterized as 9-methoxypentadecanoic acid, 9-methoxyheptadecanoic acid, 13-methoxyheneicosanoic acid and 15-methoxytricosanoic acid [39]. The following biosynthetic scheme was proposed to be operative in this alga: 9-MeO-17:0 → 13-MeO-21:0 → 15-MeO-23:0.

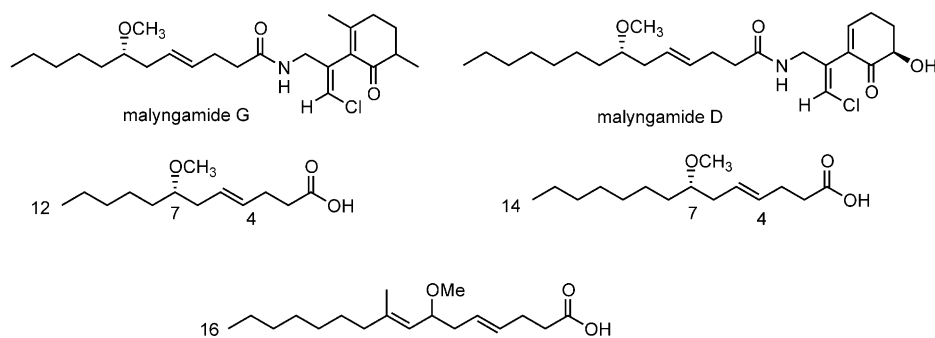


Fig. 8. Representative structures of the malyngamides and the structures of the three methoxylated fatty acids (–)-7-methoxydodec-4(*E*)-enoic acid, 7(*S*)-methoxytetradec-4(*E*)-enoic acid, and 7-methoxy-9-methylhexadeca-4(*E*),8(*E*)-dienoic acid.

Mid-chain methoxylated fatty acids are also part of the malyngamides. The malyngamides are bioactive marine metabolites mainly biosynthesized by the marine cyanophyte *Lyngbya majuscula* [40–47]. Many of the malyngamides contain bioactive methoxylated fatty acids incorporated into their structures as *N*-substituted amides. However, three of these fatty acids, namely the 7(*S*)-methoxytetradec-4(*E*)-enoic acid [45], 7-methoxy-9-methylhexadeca-4(*E*),8(*E*)-dienoic acid [43], and the recently discovered (–)-7-methoxydodec-4(*E*)-enoic acid [40] have been independently isolated (Fig. 8).

4.2. Biological studies

There are just a few isolated reports on the biological activity of saturated mid-chain methoxylated fatty acids. This is probably due to the fact that they do not seem to exhibit any significant and/or exciting biological activity. For example, when the 9- and 10-monomethoxy derivatives of stearic acid were incorporated into *Tetrahymena* cells an immediate reduction in fatty acid desaturase activity was observed [48]. This was explained on the basis of an increase in membrane fluidity due to the phospholipid-bound methoxy fatty acids, which modulated the fatty acid desaturase activity. The methoxylated compounds were incorporated into the membranes without further metabolism [48].

A monomethoxy derivative of stearic acid was prepared and shown to elicit a significant lengthening of the circadian rhythm of spore formation of the *bd csp cel* strain of the mold *Neurospora crassa* [49]. This cell mutation confers the strain a partial requirement for saturated fatty acids and perturbs the circadian rhythm of spore formation. For example, the rhythm is lengthened upon addition of *cis*-unsaturated fatty acids. In this particular experiment only the monomethoxy stearic acid was active, but the di- and trimethoxy stearic acid analogs were inactive, despite their incorporation.

The monounsaturated mid-chain methoxylated fatty acids from *L. majuscula* have exhibited the most significant biological activity. For example, the 7(*S*)-methoxytetradec-4(*E*)-enoic acid, isolated from *L. majuscula*, displayed antimicrobial activity when tested against the gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* [45]. On the other hand,

the (–)-7-methoxydodec-4(*E*)-enoic acid was non-cytotoxic to KB cells in tissue culture but showed immunosuppressive activity ($ED_{50} = 6 \mu\text{g ml}^{-1}$) on culture cells with concanavaline K and LPS [40].

4.3. Synthetic studies

Much of the earlier synthetic work with mid-chain methoxylated fatty acids aimed at locating double bonds in long-chain methyl esters by the so-called methoxymercuration–demercuration reaction followed by mass spectrometry [50,51]. In this approach a mixture of methoxylated regioisomers is obtained once the methyl esters of monounsaturated fatty acids react with mercuric acetate in methanol to produce methoxymercuriacetate adducts. Demercuration of these derivatives with sodium borohydride yields the methoxylated fatty acid methyl esters, which can be readily purified by thin-layer chromatography.

A useful alternative for the synthesis of mid-chain methoxylated derivatives of olefins, but affording lower yields, was recently proposed using borontrifluoride-methanol ($\text{BF}_3\text{-MeOH}$) [52]. For example, under these conditions methyl (*Z*)-11-tetradecenoate is partially transformed into methyl 11-methoxytetradecanoate and methyl 12-methoxytetradecanoate [52]. On the other hand, the borontrifluoride-methanol derivatization of methyl 9,12-octadecadienoate affords four monomethoxy but only two dimethoxy derivatives. The dimethoxy derivatives formed were characterized as methyl 9,12-dimethoxyoctadecanoate and methyl 10,13-dimethoxyoctadecanoate. This selectivity was explained through the formation of a common *O*-methyl-tetrahydrofuranium ion as the only intermediate under the reaction conditions [53].

Perhaps, due to their biological activity, the group of mid-chain fatty acids that has received considerable synthetic attention is that derived from *L. majuscula*. In particular, the fatty acids 7(*S*)-methoxytetradec-4(*E*)-enoic acid and (–)-7-methoxydodec-4(*E*)-enoic acid have been synthesized. In a convergent synthetic approach the synthesis by Müller et al. of 7(*S*)-methoxytetradec-4(*E*)-enoic acid started with (*R*)-(2,2-dimethyl-1,3-dioxolan-4-ylmethyl) *p*-toluenesulfonate, which was coupled with 1-hexylmagnesium bromide under Li_2CuCl_4 catalysis [54]. The resulting dioxolane was converted into the desired optically active epoxide by reaction with HBr/AcOH followed by ring closure with potassium hydroxide in methanol, which afforded the epoxide (Fig. 9). The other coupling unit was made from the reaction of 3-(2,4,10-trioxatricyclo[3.3.1.1^{3,7}]dec-3-yl)propanal with tetrabromomethane and triphenylphosphine affording the terminal dibrominated alkene which was subsequently transformed into the terminal alkyne with *n*-BuLi [54]. Coupling of the lithiated alkyne with the epoxide proceeded stereoselectively with no racemization. The resulting alkyne was then hydrogenated under Lindlar's catalyst into the *cis* double bond, which was subsequently photochemically isomerized to the *trans* double bond with phenyldisulfide [54]. Final methylation with *n*-BuLi and MeI, and hydrolysis of the orthoester group resulted in the desired 7(*S*)-methoxytetradec-4(*E*)-enoic acid (Fig. 9).

The synthesis of (–)-7-methoxydodec-4(*E*)-enoic acid by Mesguiche et al. started with oct-1-en-3-ol which was converted into 1-iodo-3-methoxyoctane by first methylating the C-3 hydroxy group followed by hydroboration of the double bond and iodination of the terminal hydroxy group [40]. On the other hand, butan-1,4-diol was monoprotected with dihydropyran and oxidized with PDC to obtain the protected butanal. A Wittig reaction between the protected butanal and the phosphorane derived from 1-iodo-3-methoxyoctane resulted in a *Z/E* mixture (75:25) of

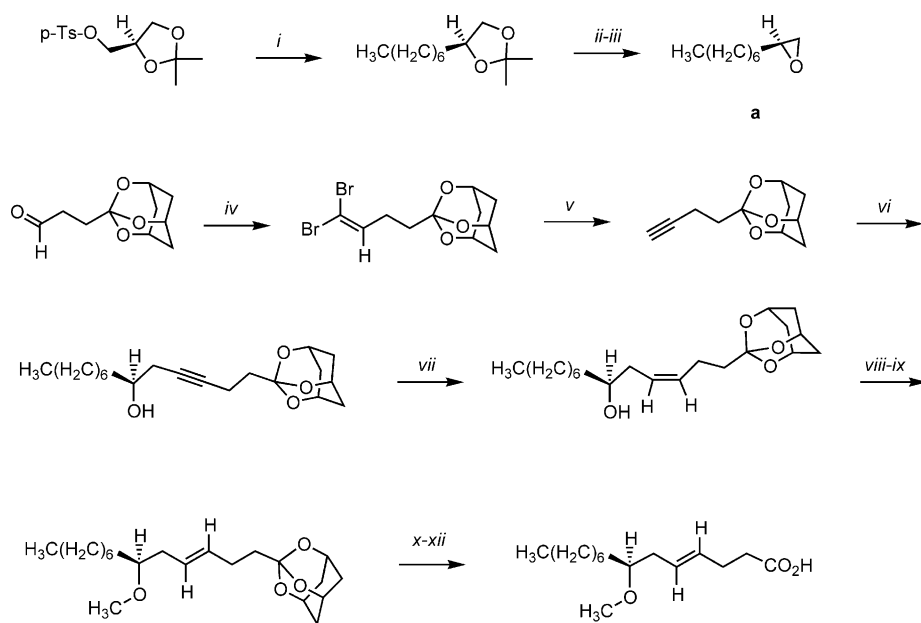


Fig. 9. Synthesis of 7(*S*)-methoxytetradec-4(*E*)-enoic acid. (i) Li_2CuCl_4 , THF, hexylmagnesium bromide; (ii) HBr/HOAc; (iii) KOH/MeOH; (iv) $\text{CBr}_4/\text{CH}_2\text{Cl}_2/\text{PPh}_3$; (v) *n*-BuLi, 2 eq.; (vi) *n*-BuLi, HMPA, *a*; (vii) $\text{H}_2/\text{Pd/C}$; (viii) hv , PhSSPh, cyclohexane; (ix) *n*-BuLi, MeI; (x) MeOH/HCl; (xi) KOH/ethanol; (xii) H_2SO_4 .

olefins. Deprotection of the alcohol with Amberlyst 15 and oxidation with PDC afforded the desired 7-methoxydodec-4(*E*)-enoic acid (Fig. 10).

5. α -Methoxylated fatty acids and derivatives

5.1. Naturally occurring

α -Methoxylated fatty acids have only been identified in the phospholipids of sponges. These α -methoxylated fatty acids share the common molecular properties of possessing the *R* configuration at the chiral center, and are basically phospholipid bound. The first naturally occurring α -methoxylated fatty acids were found in phosphatidylethanolamines and phosphatidylserines from the sponge *Higginsia tethyoides*, which contained saturated, monounsaturated, and diunsaturated α -methoxylated fatty acids with chain-lengths between 19 and 28 carbon atoms [55,56]. All of these α -methoxylated fatty acids were straight-chain fatty acids and the double bond positions in the monounsaturated fatty acids were encountered at either Δ^{17} , Δ^{18} , Δ^{19} or Δ^{21} . In addition, the very long-chain diunsaturated fatty acids 2-methoxy-5,19-hexacosadienoic acid and 2-methoxy-7,21-octacosadienoic acid were also characterized. The absolute configuration at the methoxylated carbon was determined by circular dichroism measurements on methyl 2-methoxy-7,21-octacosadienoate since a strong negative Cotton effect at about 210 nm and a weaker positive one at 235–240 nm was observed [55].

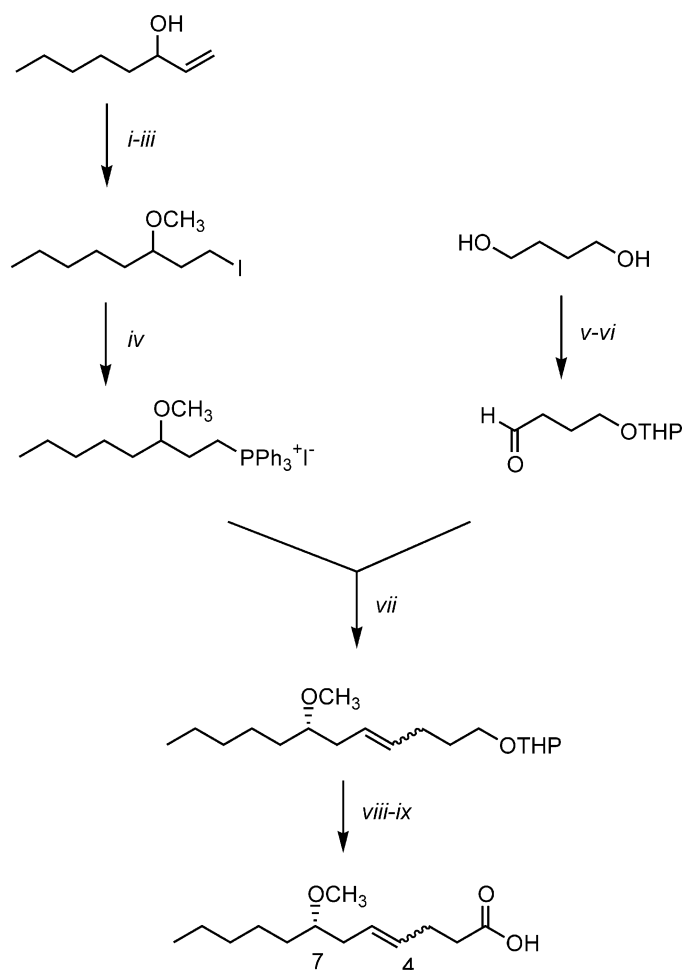


Fig. 10. Total synthesis of (–)-7-methoxydodec-4(*E*)-enoic acid. (i) NaH, CH₃I/THF reflux; (ii) BH₃THF then NaOH, H₂O₂; (iii) I₂, PPh₃, imidazole, diisopropylamine/CH₃CN, Et₂O, 25 °C; (iv) PPh₃/CH₃CN reflux; (v) DHP, Amberlyst 15 cat., 25 °C; (vi) PDC/CH₂Cl₂, 25 °C; (vii) LiHMDS/THF (–78 °C then 25 °C); (viii) Amberlyst 15 cat./MeOH, 25 °C; (ix) PDC/DMF, 25 °C.

While the first very long-chain α -methoxylated fatty acids (C₁₉–C₂₈) could have arisen from sponge cells, recent examples of short-chain analogs (C₁₄–C₁₈) are postulated to originate from bacteria in symbiosis with sponges [57–63]. A novel series of α -methoxylated fatty acids have been reported from Caribbean sponges, in particular from the genera *Amphimedon*, *Callyspongia* and *Sphaciospongia*, and includes saturated and Δ^6 monounsaturated straight-chain fatty acids with chain lengths between 14 and 18 carbons, methyl-branched *iso-anteiso* saturated and Δ^6 monounsaturated methoxylated fatty acids with chain lengths between 15 and 16 carbons, and only one single example of a Δ^5 methoxylated monounsaturated fatty acid, namely the (5*Z*)-2-methoxy-5-hexadecenoic acid (Table 1). It is interesting to emphasize here that in the short-chain (C₁₄–C₁₈)

Table 1

 α -Methoxylated fatty acids identified to date in the phospholipids^a of marine sponges

Fatty acid	Reference
2-methoxy-6-tetradecenoic (2-OMe- Δ^6 -14:1)	[62]
2-methoxytetradecanoic (2-OMe-14:0)	[62]
2-methoxy-13-methyl-6-tetradecenoic (2-OMe- <i>i</i> - Δ^6 -15:1)	[62]
2-methoxy-13-methyltetradecanoic (2-OMe- <i>i</i> -15:0)	[61]
2-methoxy-6-pentadecenoic (2-OMe- Δ^6 -15:1)	[62]
2-methoxypentadecanoic (2-OMe-15:0)	[62]
2-methoxy-14-methylpentadecanoic (2-OMe- <i>i</i> -16:0)	[61]
2-methoxy-13-methylpentadecanoic (2-OMe- <i>ai</i> -16:0)	[61]
2-methoxy-5-hexadecenoic (2-OMe- Δ^5 -16:1)	[57]
2-methoxy-6-hexadecenoic (2-OMe- Δ^6 -16:1)	[57]
2-methoxyhexadecanoic (2-OMe-16:0)	[60]
2-methoxy-6-heptadecenoic (2-OMe- Δ^6 -17:1)	[63]
2-methoxy-6-octadecenoic (2-OMe- Δ^6 -18:1)	[63]
2-methoxyoctadecanoic (2-OMe-18:0)	[62]
2-methoxynonadecanoic (2-OMe-19:0)	[56]
2-methoxyicosanoic (2-OMe-20:0)	[56]
2-methoxyheneicosanoic (2-OMe-21:0)	[56]
2-methoxydocosanoic (2-OMe-22:0)	[56]
2-methoxy-16-tricosenoic (2-OMe- Δ^{16} -23:1)	[56]
2-methoxytricosanoic (2-OMe-23:0)	[56]
2-methoxy-17-tetracosenoic (2-OMe- Δ^{17} -24:1)	[56]
2-methoxytetracosanoic (2-OMe-24:0)	[56]
2-methoxy-18-pentacosenoic (2-OMe- Δ^{18} -25:1)	[56]
2-methoxy-5,9-hexacosadienoic (2-OMe- $\Delta^{5,9}$ -26:2)	[58]
2-methoxy-5,19-hexacosadienoic (2-OMe- $\Delta^{5,19}$ -26:2)	[56]
2-methoxy-19-hexacosenoic (2-OMe- Δ^{19} -26:1)	[56]
2-methoxy-20-heptacosenoic (2-OMe- Δ^{20} -27:1)	[56]
2-methoxy-7,21-octacosadienoic (2-OMe- $\Delta^{7,21}$ -28:2)	[55]
2-methoxy-21-octacosenoic (2-OMe- Δ^{21} -28:1)	[56]

^a Mainly identified in phosphatidylethanolamine and phosphatidylserine.

series the Δ^6 monounsaturations predominate, with the only exception of the (5*Z*)-2-methoxy-5-hexadecenoic acid [57].

The biosynthesis of the α -methoxylated fatty acids has not been elucidated so far, but it is possible that they arise from the corresponding α -hydroxylated fatty acids (Fig. 11). At what stage of the biosynthesis the double bond incorporated into the acyl chain remains a matter of speculation, but a good possibility (based on the number of saturated analogs isolated so far) is desaturation after the α -methoxy functionality is in place. On the basis of these assumptions we can now begin to postulate a series of unprecedented fatty acid biosynthetic pathways for these methoxylated fatty acids. Our postulated fatty acid biosynthetic sequence for most of the normal even-chain α -methoxylated fatty acids reported to date is shown in Fig. 11. This series is probably the most complete, but no single organism can do all of the biosynthetic routes shown. The biosynthetic sequence in Fig. 11 starts with myristic acid (14:0).

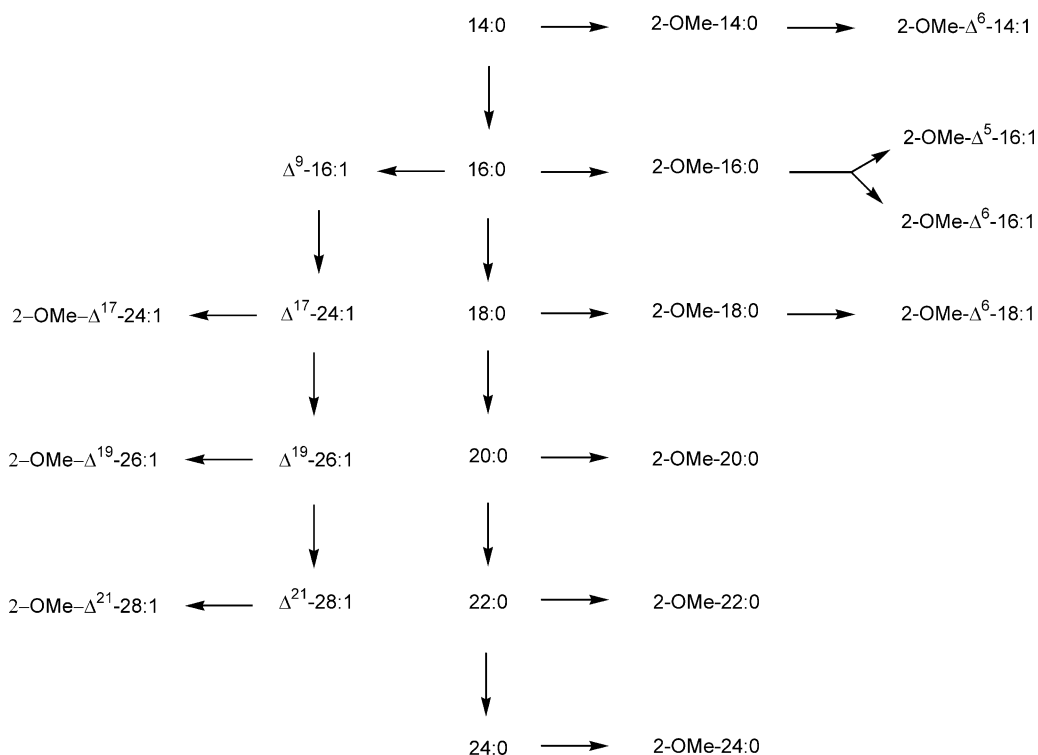


Fig. 11. Postulated biosynthesis of normal even-chain methoxylated fatty acids.

5.2. Biological studies

There is little information on the biological activity of α -methoxylated fatty acids since many of these fatty acids are now beginning to emerge. The two α -methoxy fatty acids (*Z*)-2-methoxy-6-hexadecenoic acid and (*Z*)-2-methoxy-5-hexadecenoic acid displayed similar antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* (MIC 0.35 $\mu\text{mol/ml}$) and *Streptococcus faecalis* (MIC 0.35 $\mu\text{mol/ml}$) but are inactive against Gram-negative bacteria [59]. The saturated precursor 2-methoxyhexadecanoic acid was not antimicrobial in this bioassay.

5.3. Synthetic studies

The first synthesis for the (*Z*)-2-methoxy-6-hexadecenoic acid, as the methyl ester, was reported by Soderquist et al. [64]. The key for the construction of the α -methoxy functionality was the addition of a carboxy synthon to the corresponding aldehyde, in this case (*Z*)-5-pentadecenal, followed by methylation of the hydroxy group. This synthesis started with the Suzuki-Miyaura cross coupling of 4-bromo-1-butyl-9-borabicyclononane with (*Z*)-1-bromo-1-undecene, prepared as shown in Fig. 12. The resulting 5-pentadecen-1-ol was oxidized with PCC to 5-pentadecenal. This aldehyde was reacted with tris(methylthio)methylithium, affording the corresponding α -hydroxy ortho(trithio)ether, which in turn it was methylated in situ using NaH/DMF and MeI

to obtain the corresponding methoxylated adduct (Fig. 12). Final HgCl_2/HgO catalyzed hydrolysis afforded the desired methyl (*Z*)-2-methoxy-6-hexadecenoate, which confirmed the structure of the natural methyl ester.

A more recent synthesis utilized Mukaiyama's trimethylsilyl cyanide addition to aldehydes as the key step to introduce the carboxy functionality [59,65]. Therefore, this synthesis also required the preparation of (*Z*)-5-pentadecenal as the key intermediate (Fig. 13). In this case, commercially available decyl aldehyde was coupled with 4-carboxybutyltriphenylphosphonium bromide, under Wittig conditions, resulting in a 10:1 mixture of the known (*Z*)- and (*E*)-5-pentadecenoic acids. The acids were then reduced to the desired (*Z*)-5-pentadecenal via (*Z*)-5-pentadecen-1-ol, a known pheromone. Addition of trimethylsilyl cyanide, under triethylamine catalysis, to (*Z*)-5-pentadecenal yielded 2-trimethylsilyloxy-6-hexadecenitrile. The trimethylsilyl cyanide was then transformed into the intermediate α -hydroxy amide with concentrated HCl, and then hydrolyzed to (*Z*)-2-hydroxy-6-hexadecenoic acid with 50% NaOH. Double methylation was

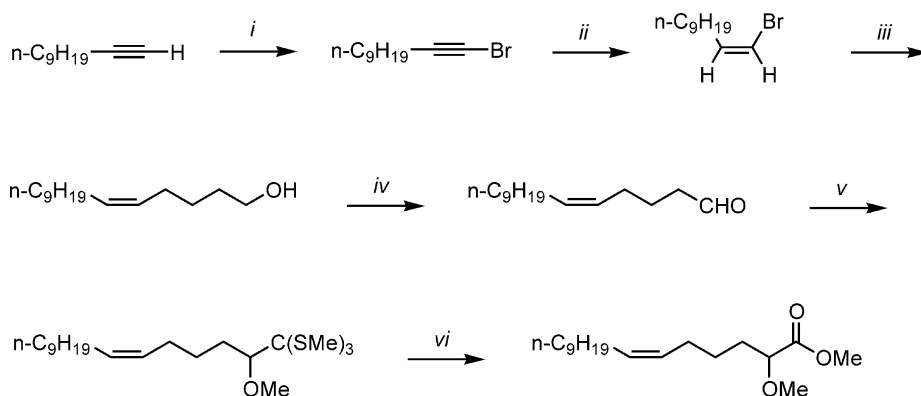


Fig. 12. Soderquist synthesis of methyl (*Z*)-2-methoxy-6-hexadecenoate. (i) NBS/ Me_2CO , AgNO_3 ; (ii) 9-BBN-H, HOAc, $\text{HO}(\text{CH}_2)_2\text{NH}_2$; (iii) 4-bromo-1-butyl-9-borabicyclononane/ NaOH , THF/ $\text{Pd}(\text{PPh}_3)_4$; (iv) PCC/ CH_2Cl_2 ; (v) $\text{LiC}(\text{SMe})_3/\text{H}_2\text{O}/\text{NaH}/\text{MeI}$; (vi) $\text{HgCl}_2/\text{HgO}/\text{MeOH}/\text{H}_2\text{O}$.

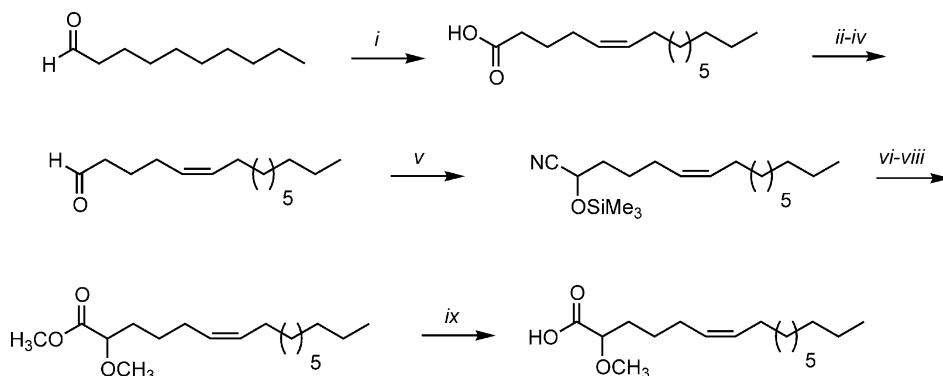


Fig. 13. Carballeira's synthesis of (*Z*)-2-methoxy-6-hexadecenoic acid. (i) $\text{HO}_2\text{C}(\text{CH}_2)_3\text{CH}_2\text{PPh}_3^+\text{Br}^-$, *n*-BuLi, THF/DMSO (1:1), -10°C ; (ii) 1N HCl–MeOH, 3 h; (iii) LiAlH_4 –THF, -78°C ; (iv) PCC (1.5 eq.) CH_2Cl_2 , rt; (v) TMS-CN, Et_3N , CH_2Cl_2 , -10°C , 2 h; (vi) HCl (conc.) rt; (vii) 50% NaOH, heat; (viii) NaH/DMSO , CH_3I ; (ix) KOH–EtOH.

successfully accomplished with NaH and methyl iodide, in DMSO, resulting in the ester, which was saponified with KOH in ethanol affording the desired acid.

The synthesis of (*Z*)-2-methoxy-5-hexadecenoic acid was done in a similar fashion, but it required the preparation of (*Z*)-4-pentadecenal first [59]. In this case, this was accomplished starting with commercially available 1-dodecyne, which was coupled with 2-(2-bromoethyl)-1,3-dioxolane and *n*-BuLi in tetrahydrofuran-hexamethylphosphoramide, resulting in the expected 2-(3-tetradecyne)-1,3-dioxolane, Fig. 14. Subsequent catalytic hydrogenation, using Lindlar's catalyst, afforded 2-(3-tetradecenyl)-1,3-dioxolane. The dioxolane was removed with 5% HCl in acetone–water (1:1), and the equilibrium favored (*Z*)-4-pentadecenal. Addition of trimethylsilyl cyanide to (*Z*)-4-pentadecenal, under triethylamine catalysis as described by Mukaiyama for other shorter-chain analogues [65], resulted in 2-trimethylsilyloxy-5-hexadecenitrile. Under basic conditions the trimethylsilyloxynitrile easily reverts to the original aldehyde. Therefore, the trimethylsilyloxynitrile had to be first transformed into the corresponding α -hydroxy amide under concentrated acid conditions (HCl), and then hydrolyzed to the α -hydroxy acid with 50% NaOH. Under these conditions the intermediate (*Z*)-2-hydroxy-5-hexadecenoic acid was obtained. Double methylation was then successfully accomplished with NaH and methyl iodide in DMSO resulting in methyl (*Z*)-2-methoxy-5-hexadecenoate. Final saponification with KOH in ethanol afforded the desired (*Z*)-2-methoxy-5-hexadecenoic acid.

Despite the fact that all of the above syntheses resulted in racemic 2-methoxy acids, the stereospecific synthesis of the naturally occurring (*R*)-2-methoxyhexadecanoic acid was also recently reported [66]. This synthesis started with commercially available (\pm)-2-hydroxyhexadecanoic acid for which the *S* enantiomer was selectively acetylated (47% yield out of a maximum 50%, > 95% ee) with vinyl acetate in THF using the lipase *Pseudomonas fluorescens* (Fig. 15). In order to facilitate the separation of the acetylated acid from the non-acetylated acid, both compounds

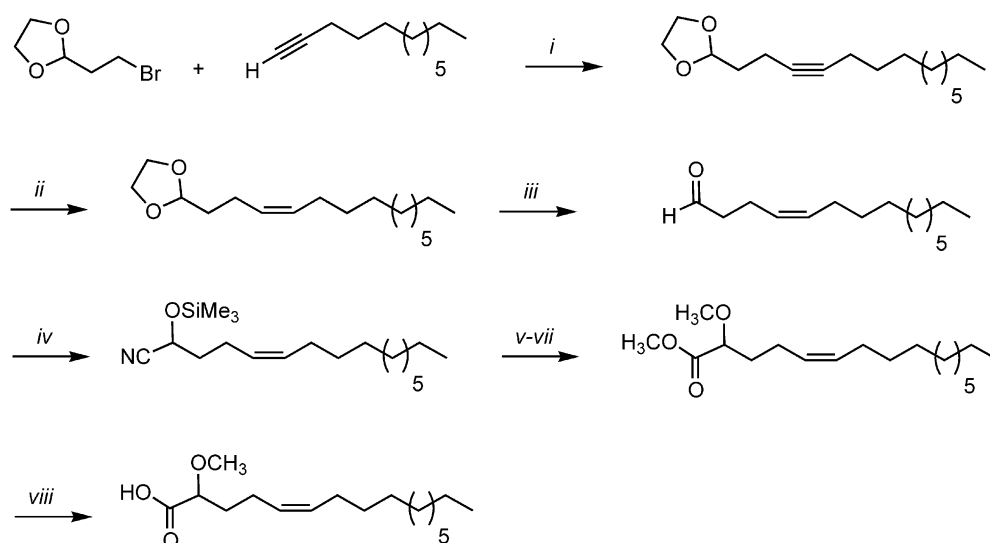


Fig. 14. Synthesis of (*Z*)-2-methoxy-5-hexadecenoic acid. (i) *n*-BuLi, THF-HMPA, -70°C ; (ii) H_2 , Lindlar; (iii) 5% HCl, $\text{Me}_2\text{CO}-\text{H}_2\text{O}$, 60°C ; (iv) TMS-CN, Et_3N , CH_2Cl_2 , -10°C ; (v) HCl conc. rt; (vi) 50% NaOH, heat; (vii) NaH/DMSO, CH_3I ; (viii) KOH-EtOH.

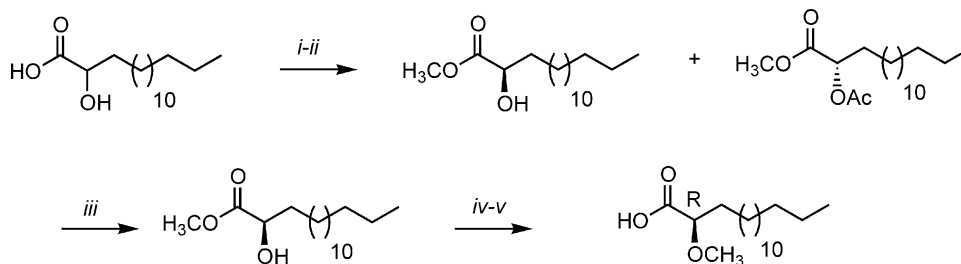


Fig. 15. Synthesis of (*R*)-2-methoxyhexadecanoic acid. (i) *Pseudomonas fluorescens*, $\text{CH}_2=\text{CHOAc}$, BHT, THF; (ii) CH_2N_2 , ether; (iii) silica gel column chromatography; (iv) NaH, MeI, DMSO; (v) KOH/ethanol.

were methylated with diazomethane, mild conditions that avoided cleavage of the acetate functionality. The methyl (*R*)-2-hydroxyhexadecanoate and the methyl (*S*)-2-acetoxyhexadecanoate were then separated by silica gel column chromatography. The purified methyl (*R*)-2-hydroxyhexadecanoate was further methylated with NaH/MeI in DMSO, and finally saponified with KOH in ethanol, which afforded the (*R*)-2-methoxyhexadecanoic acid with good enantiomeric purity (>95% ee). This represented the first synthesis for the (*R*)-2-methoxyhexadecanoic acid [66].

6. Summary

The chemistry of methoxylated lipids continues to attract much attention. The antitumor properties of 1-*O*-(2-methoxyhexadecyl)glycerol, and its phosphocholine derivatives, has been further explored in recent years. In this literature review we have observed that among the unsaturated naturally occurring methoxylated alkyl glycerol lipids, mainly of marine origin, Δ^4 monounsaturated alkyl chains predominate. In addition, the methoxy group has always been found at C-2 in the alkyl chain, bespeaking of a common biosynthetic origin with the α -methoxylated fatty acids.

There are almost no naturally occurring ω -methoxylated fatty acids. Most of these compounds have been synthesized as myristic acid analogs so as to interfere with the *N*-myristoyltransferase (NMT) of viruses and fungi. In particular the 12-methoxydodecanoic acid, either alone or as part of a phospholipid or as a component of a prodrug, has been amply studied for its antiviral and antifungal properties. Further research in this area could lead to useful therapeutic candidates.

Marine organisms have provided most of the bona fide mid-chain methoxylated and α -methoxylated fatty acids. In the family of mid-chain methoxylated fatty acids the unsaturated methoxylated fatty acids from *Lynbya majuscula* continue to attract attention for their antimicrobial properties and two syntheses have been reported. A recent finding of several saturated mid-chain methoxylated fatty acids from *Schizymania dubyi* point to the existence of a previously unexplored fatty acid biosynthetic pathway in algae. In addition, several novel fatty acid biosynthetic pathways can now be postulated to exist in sponges since a complete series of α -methoxylated fatty acids with chain-lengths between 14 and 28 carbon atoms has been discovered. In contrast to the methoxy alkyl glycerols in the C_{14} – C_{18} series of monounsaturated α -methoxylated fatty acids the Δ^6 monounsaturations predominate. Initial bioassays indicate that these compounds

have potential as antimicrobial agents, but the full potential of the α -methoxylated fatty acids as therapeutic candidates remains to be elucidated.

Acknowledgements

We are indebted to Professor R.G. Ackman (Dalhousie University) who initially called our attention to the 2-methoxy glycerol ethers found by Hallgren et al. in a wide variety of organisms and organs. The support of the National Institutes of Health (grant no. SO6GM08102) to NMC, which made possible the discovery of many of the α -methoxylated fatty acids, is greatly appreciated.

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