Some Recent Developments in the Isolation and Synthesis of Lipids

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Abstract

The isolation of certain lipids in pure form from complex natural mixtures, and improved procedures for the synthesis of fatty acids and their esters, of synthetic intermediates, of alkoxy-lipids, and of some other unusual lipids are reviewed.

Introduction

Lipids occur in nature as complex mixtures of different compound classes. It has become apparent that some individual molecular species of the various classes may have important biological functions and may be involved intimately in metabolism, whereas most of the lipids may fulfill only passive structural roles. Thus, there is a need for pure individual lipid compounds which can be used in biochemical, biophysical and metabolic studies. New analytical techniques have revealed shortcomings of many procedures for the preparation of lipids, and have facilitated development of improved methods of isolation and new routes of synthesis.

There will be described some recent work on the preparation of neutral lipids, in forms in which they occur in nature, or in which they play roles as intermediates for chemical synthesis or biosynthesis of more complex compounds. Emphasis will be given to studies that have been conducted at the The Hormel Institute. However, reference will be made also to comprehensive reviews on specialized subjects.

Materials and Methods

Isolation and Synthesis of Fatty Acids and Their Esters

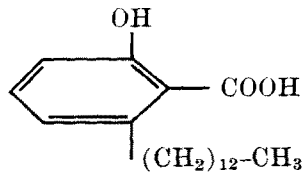
The most common starting materials for the preparation of a wide variety of lipids are fatty acids that are isolated from natural sources. Pure saturated and unsaturated fatty acids of different chain-lengths, which are made available by the Lipids Preparation Laboratory of The Hormel Institute, have facilitated the synthetic studies in our laboratories.

Methods for the isolation of fatty acids include distillation, low-temperature crystallization, chromatography and countercurrent distribution. Using combinations of these techniques, we have developed improved procedures for the preparation of, e.g., petroselinic acid from parsley seed oil (1), arachidonic acid from hog liver (2), and pentaenoic and hexaenoic acids of C_{20} and C_{22} chain-lengths from beef liver (3) and, more recently, from fish oils (4). Odd-numbered fatty acids have been isolated from mullet, *Mugil cephalus* (5,6).

Acids uniformly labeled with ^{14}C have been prepared from cultures of the alga *Chlorella pyrenoidosa* which were grown in an atmosphere of ^{14}C-carbon dioxide (7), and also from the protozoon *Ochromonas danica* propagated on a medium containing ^{14}C-acetate (8). Several of these acids have been valuable in metabolic studies discussed in an earlier review (9).

Cyclopentenylic acids, such as hydnocarpic, chaulmoogric and gorlic acids, have been isolated from the seeds of *Hydnocarpus wightiana* by a combination of solid-liquid countercurrent distribution with urea, and subsequent liquid-liquid countercurrent distribution (10).

Reversed-phase partition chromatography in columns has been used for the isolation of anacardic acids such as [I] from the leaves of ginkgo and from cashew nuts (11).



[I]

Classical procedures of fatty acid synthesis, such as chain elongation based on the reaction of alkyl halides with potassium cyanide or sodium malonate, are not satisfactory when applied to long-chain compounds, especially polyunsaturated ones (12). Methanesulfonates (mesylates) (13) proved to be superior to halides and tosylates as intermediates for the preparation, with excellent yields, of unusual unsaturated fatty acids from naturally occurring compounds (14). The chemical synthesis of all *cis,cis*-octadecadienoic acids (15) having methylene-interrupted systems of double bonds was facilitated by the use of mesylates. Similarly, some of the isomers of linoleic acid and other polyunsaturated acids labelled with ^{14}C in positions 1 or 3 have been prepared (16) for use in enzymatic studies of fatty acid biosynthesis. The Favorsky rearrangement of haloketones proved to be useful for the preparation of saturated and unsaturated 2-methyl fatty acids (17).

Individual species of pure triglycerides containing cyclopentenylic acids, viz., trihydnocarpin, hydnocarpodichaulmoogrin, chaulmoogrodihydnocarpin and trichaulmoogrin have been isolated from maratti oil (10). An optically active lipid has been isolated from the seed oil of the Chinese tallow tree, *Sapium sebiferum*, and was shown to be an estolide triglyceride (18).

Unsaturated mono-, di- and triglycerides (19) may be prepared by esterification of trityl glycerol or dityrityl glycerol with brominated acid chlorides, followed by acid-catalyzed cleavage of the trityl ether linkage, and debromination of the acyl moieties (20). However, debromination leads to the formation of some *trans*-unsaturated compounds (21).

α-Monoglycerides, α,α'-diglycerides, and triglycerides can be synthesized by a method in which the glycerol moiety is built up stepwise and the alcoholic groups become available successively to react with different fatty acids (22). An acylated glycolic acid chloride is reacted with diazomethane, and hydrolysis of the resulting diazo compound with perchloric acid leads to a monoester of dihydroxyacetone. Catalytic reduction of this ester yields an α-monoglyceride, whereas acylation and subsequent catalytic reduction yields an α,α'-diglyceride.

Mixed acid triglycerides can be obtained by esterification of the mono- and diglycerides. This method is applicable to the preparation of glycerides labeled with one, two or three ^{14}C-atoms in the glycerol moiety, or in one, two or three of the acyl groups, or in both glycerol and fatty acid moieties (22). Unfortunately, such synthesis is not suitable for the preparation of certain unsaturated glycerides because it involves catalytic hydrogenation.

The transesterification of long-chain alkyl acetates and cholesteryl acetate with methyl esters of fatty acids has been used for the preparation of wax esters and cholesteryl esters (23), respectively.

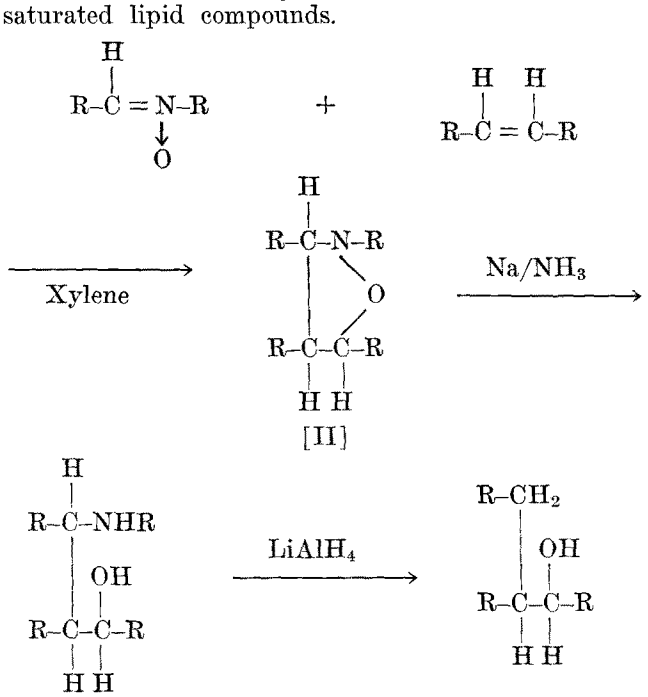
Intermediates in Lipid Synthesis

Until recently, acyl chlorides and alkyl halides were the intermediates utilized most frequently for the preparation of more complex lipids. For a variety of reactions, esters of long-chain alcohols with methanesulfonic acid, i.e., mesylates, are superior to halides and tosylates (13,24). Many reactions of long-chain mesylates proceed quantitatively and without alteration of double bonds, and make possible syntheses of hitherto unavailable lipids.

Hydrocarbons such as *cis,cis*-octadecadiene have been obtained in quantitative yields, by reducing the corresponding mesylates with lithium aluminum hydride (13). Alkyl bromides such as *cis,cis*-octadecadienyl bromide have been prepared by the quantitative reaction of long-chain mesylates with anhydrous magnesium bromide in diethyl ether (25). Nitriles, which can be converted to amines, amides or acids, may also be prepared via mesylates (14). Saturated and unsaturated aldehydes which previously were prepared by a modified Grundmann synthesis (26) have been obtained, more conveniently, by oxidation of mesylates with dimethyl sulfoxide (27).

In addition, mesylates may serve as alkylating agents (see Table I) in the preparation of naturally occurring alkoxylipids, such as alkyl ethers of glycerol and other polyhydric alcohols.

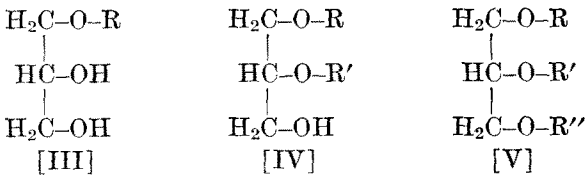
Long-chain alcohols and amino alcohols have been prepared (28) by reduction of isoxazolidines [II] which were obtained by addition of nitrones to unsaturated lipid compounds.



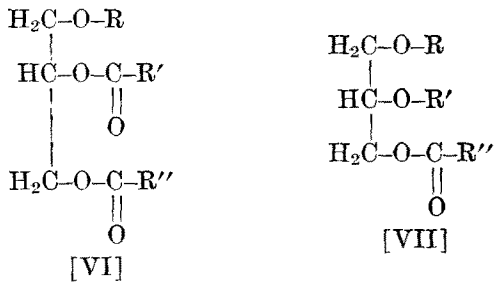
Isolation and Synthesis of Unusual Lipids

Alkyl glycerol-(1) ethers and dialkyl glycerol-(1,2) ethers have been obtained through alkaline hydrolysis of lipids from human and animal tissues and from microorganisms (29,30). Hydrogenolysis with lithium aluminum hydride is superior to saponification, especially when small amounts of glycerol ethers are to be isolated (31,32).

Individual alkyl glycerol-(1) ethers [III] may be prepared by reacting mesylates with the potassium salt of isopropylidene glycerol, followed by hydrolysis of the isopropylidene alkyl glycerol ether (24). The synthesis of saturated and unsaturated dialkyl glycerol-(1,2) ethers [IV] having identical or different alkyl moieties has also been reported (33). Although trialkyl glycerol ethers [V] have not been found in nature as yet, they are of interest as structural analogs of triglycerides and because they are not hydrolyzed by lipases (34). Trialkyl glycerol ethers may be synthesized (33) by further alkylation of alkyl glycerol-(1) ethers and dialkyl glycerol-(1,2) ethers.



Alkyl diglycerides [VI] have been isolated from human fats (35) and, in larger amounts, from shark liver oils (32,36), but dialkyl glycerides [VII] have not been found in nature.



Alkyl diglycerides as obtained from natural sources have been identified only as lipid classes but not as individual compounds. The occurrence of a large variety of glycerol ethers having odd numbered or branched alkyl groups, e.g., dihydrophytyl, created a need for pure synthetic compounds which can be used as reference materials. Further, individual alkyl diglycerides and dialkyl glycerides of known structure are useful model substances for studying the enzymatic hydrolysis of both long chain ethers and esters.

TABLE I

Reactions of Aliphatic Methanesulfonates
$R-CH_2-O-SO_2-CH_3$

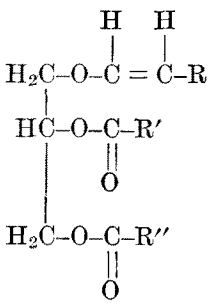
Experimental conditions	Products	Yield %	(Ref.)
$LiAlH_4$ → Diethyl ether, 35 C, 6 hr.	Hydrocarbons	>95	(13)
$MgBr_2$/Diethyl ether → Diethyl ether, 20 C, 24 hr.	Bromides	>95	(25)
KCN → Dimethyl sulfoxide, 80 C, 1 hr.	Nitriles	>95	(14)
Dimethyl sulfoxide, $NaHCO_3$ → 170 C, 10 min.	Aldehydes	60–70	(27)
Isopropylidene glycerol-K → Xylene, 140 C, 4 hr.	Alkyl glycerol ethers	80–90	(24,38)

Systematic investigations of the influence of chain length and unsaturation of the lipid substrates will provide additional understanding of the specificities and mechanisms of etherase and lipase activities.

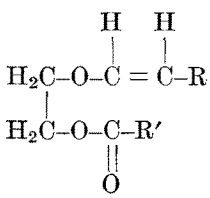
A series of alkyl glycerides [VI and VII] has been prepared by acylation of synthetic alkyl glycerol ethers and dialkyl glycerol ethers (37,38). The absolute configuration of natural alkyl diglycerides and alk-l-enyl diglycerides (neutral plasmalogens) [VIII] was determined by comparison with a synthetic preparation of an optically active alkyl diglyceride (38).

Alk-l-enyl glycerol ethers have been isolated via lithium aluminum hydride reduction of the total lipids of bovine heart (39) and ratfish liver (32), in which they occur as alk-l-enyl diglycerides and alk-l-enyl acyl phosphatides (plasmalogens). Neutral plasmalogens have been isolated also from these tissues by adsorption chromatography (32,40).

Esters and alk-l-enyl ether esters of ethanediol [IX] and other short-chain diols have recently been detected as constituents of animal and vegetable fats (41). However, these compounds have not been isolated. Monoesters and diesters of ethanediol and propanediols have been prepared and methods for their isolation and characterization have been developed (42).



[VIII]



[IX]

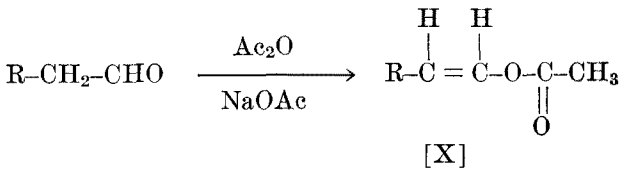
Alkyl ethers of diols have not been found in nature, probably because the procedures employed for the analysis of diol lipids are not suitable for the detection of these compounds. However, considering the chemical and the most probable metabolic relationships between alk-l-enyl ethers and alkyl ethers of glycerol, the occurrence of alkyl ethers of diols in nature may be expected.

Alkyl ethers of ethanediol have been prepared by glycol cleavage of the corresponding glycerol ethers and subsequent lithium aluminum hydride reduction of the resulting alkoxy acetaldehydes (42); dialkyl glycol ethers were obtained by further alkylation.

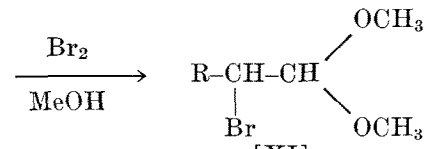
Cleavage of natural alk-l′-enyl glycerol-(1) ethers with sodium periodate in pyridine, and subsequent reduction with lithium aluminum hydride yielded alk-l-enyl glycol ethers (39). In addition, alk-l-enyl glycol ethers were synthesized (43) from aldehydes via enol acetates [X], α-bromo dimethyl acetals [XI], and 2-(α-bromo-alkyl)-1.2-dioxalanes [XII]. This sequence of reactions led to a mixture of *cis* and *trans* alk-l-enyl glycol ethers [XIII]. Acylation of these compounds yielded neutral diol plasmalogens [IX] which were resolved into the pure *cis* and *trans* isomers by argentation chromatography (43).

The diol lipids synthesized in our laboratories are now being used in studies aimed at evaluation of the nutritional effects of diol lipids and at elucidation of their metabolism.

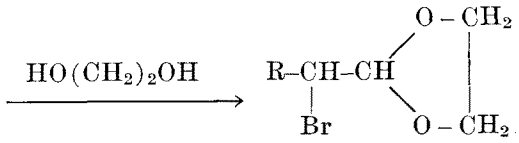
Studies of the isolation and systematic synthesis



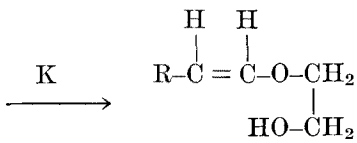
[X]



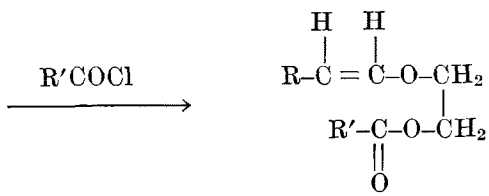
[XI]



[XII]



[XIII]



[IX]

of pure lipid compounds including labeled lipids (44) are being continued at The Hormel Institute because it is evident that a very important avenue for further progress in lipid biochemistry and molecular biology involves research on the biological behavior of individual lipid species.

ACKNOWLEDGMENT

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