THE STRUCTURE OF LIPIDS

D. CHAPMAN

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by Spectroscopic and X-Ray Techniques

With a chapter on Separation Techniques including thin layer and gas liquid chromatography

D. CHAPMAN
University Chemical Laboratory, Cambridge

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Preface

Interest in the study of lipids has intensified considerably in the last t... years. Much of this interest has arisen because of the increasing concern over the causes of atherosclerosis and other lipid diseases. At the same time general interest in the biochemical reactions of fat metabolism and in the association of lipids and proteins has also grown considerably. Interest in the lipoproteins springs from many directions. They are thought to occur in cell membranes, to be involved in lipid transport in blood and are considered to be important in such various areas as neurological, physiological, biochemical and agricultural research. Enzyme activity in mitochondria has also been associated with lipid–protein interactions.

The lipid molecules are interesting molecules in their own right and exhibit many unusual properties. Many of them have more than one melting point and some have as many as four or five. This is associated with polymorphism and is related to the variety of ways in which hydrocarbon chains can pack together. Some lipids form liquid crystals where part of the molecule is in a liquid condition one hundred degrees or more below the true melting point. In the presence of water, lipids with polar head groups aggregate to form micelles which can also be of considerable biological importance.

In this book I have set out to show the kind of information which the different modern spectroscopic and X-ray techniques can provide about lipid molecules. To do this I have discussed first the simple lipids such as the monocarboxylic acids, long chain esters and alcohols, then glycerides, phospholipids and, finally, the natural lipoproteins, such as those which occur in the myelin sheath of nerve fibres. The particular feature of the techniques discussed is in the main that they are non-destructive and, in many cases, enable the biological material itself to be studied. It is certain that a great deal of our future understanding of the role of lipid molecules in biological systems will spring from the use of these techniques.

I have included in the first chapter a brief account of modern separation methods as the combination of these with the spectroscopic techniques will be particularly powerful. In a final chapter techniques other than X-ray and spectroscopic ones are also briefly mentioned.

The book is intended as a practical book for those carrying out research on lipids, but particularly for those carrying out research on their biophysical, biochemical and medical aspects.

D. CHAPMAN

Cambridge, 1964

Acknowledgements

I wish to thank the Master and Fellows of Gonville and Caius College, Cambridge, for their award of the Comyns-Berkeley Bye-Fellowship which enabled me to work in the University Chemical Laboratory, Cambridge. This gave me the opportunity to work in what was for me a number of new scientific areas. At the same time I began to appreciate much more the growing need in biochemical and medical research for basic physical knowledge on the cumbersome awkward lipid molecules on which I had previously worked. I have always found them interesting and rather fascinating but had previously considered them to be mainly of technological importance.

I also wish to thank colleagues at the University Chemical Laboratory and also the various Unilever laboratories, in particular Professor V. M. Clark and Professor G. Dijkstra, for useful discussions, and the secretarial assistance of the staff of these laboratories.

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1. Introduction

The field of lipids

The field of lipids (lipides) is usually poorly defined, sometimes being associated only with a common solubility property. Substances ranging from the simple fatty acids and esters to complex sphingolipids, and from sterols, steroids to vitamins and colouring matters and flavours are often classified in this field. The definition more frequently accepted is that the term 'lipid' covers the esters of long-chain fatty acids and alcohols and closely related derivatives. As even this definition is somewhat vague we shall now outline more precisely the area and different classes of substance which we intend to discuss before considering the use of spectroscopy and other techniques for their study.

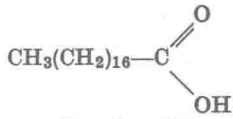
There is at present no internationally accepted system for nomenclature of these compounds and hence only the commonly used terms or most reasonable terms are adopted in this book.

FATTY ACIDS

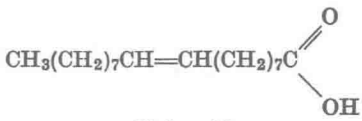
The fatty acids are a basic unit in many lipid molecules and markedly determine their properties. In nature they occur only in small quantities in a free form, e.g. the lower fatty acids such as acetic, butyric and caproic occur in free form in milk fats. Whilst it was at one time considered that free fatty acids present in tissue extract were due to degradative action undergone in isolation procedures, it has now been established that free fatty acids are normal constituents of the tissue lipid pool.

The fatty acids found in the majority of mammalian lipids are straight chain, even numbered monocarboxylic, and the most predominant of these are lauric, myristic, palmitic and stearic acids. Small amounts of branched and odd numbered acids do occur naturally but only in small percentages. Unsaturated acids are particularly important and monoenoic (oleic acid), dienoic (linoleic acid), trienoic (linolenic acid) and tetraenoic acid (arachidonic acid) are known. Linoleic acid (*cis-cis* linoleic acid) is regarded as being particularly important in biology, and it and arachidonic acid are termed 'essential fatty acids'. The latter is

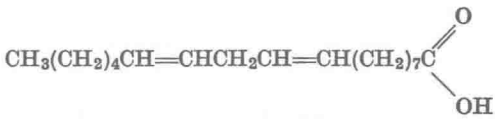
found in liver, brain and depot lipids. Some of the variations on the fatty acid structure are:



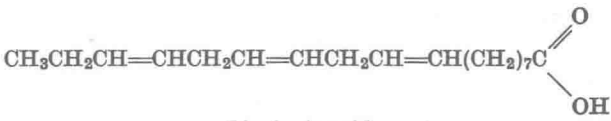
Stearic acid



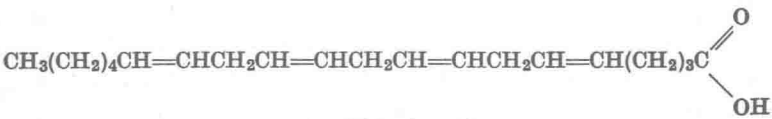
Oleic acid



Linoleic acid



Linolenic acid



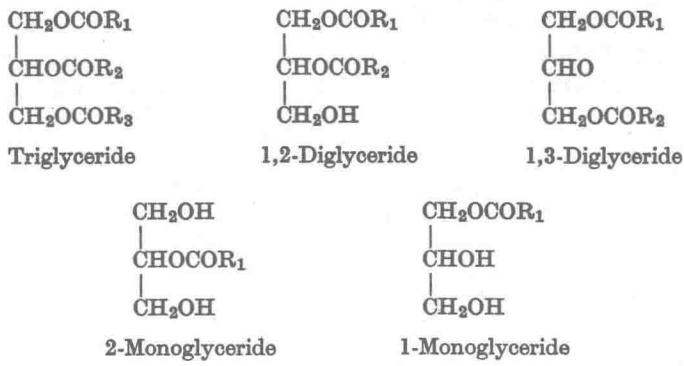
Arachidonic acid

SIMPLE LIPIDS

The simple lipids are amongst the most abundant fatty acid derivatives found in nature and exist in animal and plant tissue. The most predominant members of this class are the esters of the fatty acids with glycerol and also with cholesterol.

Glycerides

Five different derivatives are possible on esterification of glycerol with fatty acid and these are:



where R_1, R_2 and R_3 are the long-chain hydrocarbon units of the fatty acids and may differ in length and in degree of unsaturation.

In mammalian tissues triglycerides are present to the largest extent, but diglycerides and monoglycerides are also present in certain tissues. The glycerides constitute well over 98% of the lipids of the adipose tissue of the mammal, 30% of the plasma and liver lipids, and less than 10% of the red blood cell lipid. There is the possibility of a number of permutations and combinations of fatty acids on the glycerol residue. Thus two different fatty acids, R_1 and R_2, can give rise to any one of six triglycerides, whilst three fatty acids, R_1, R_2 and R_3, can give eighteen triglycerides.

The mode of distribution of the fatty acids on the glycerol molecule in natural mixtures of glycerides is still not fully understood and many theories have been proposed and criticized relating to this. Thus there is a rule of even distribution, i.e. that each of the individual acids of a given glyceride tends to be distributed as evenly as possible amongst all the glyceride molecules. (An acid has to constitute greater than two-thirds of the total acid content before it can form any appreciable amounts of a monoacid-type triglyceride.) There is also a rule of random distribution, partial random distribution and a rule of restricted random distribution.

The diglycerides and monoglycerides can also undergo acyl migration or *trans* esterification. Thus the 2-monoglycerides can be converted predominantly to the 1-isomer whilst the 1,2-diglycerides can be converted to a predominance of the 1,3-isomer.

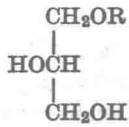
Cholesterol and cholesterol esters

Whilst glycerol is by far the major polyhydroxy alcohol found esterified to long-chain fatty acids in mammals, another important alcohol is cholesterol. This is the only sterol found associated with long-chain fatty

acids in mammals. There is particular interest in cholesterol esters at the present time because of their possible relation to atherosclerosis. There are three main sites of location of these esters, the adrenals, liver and plasma. Some uncertainty exists with regard to the fatty-acid composition of certain of these esters. Thus the lipid fractions present in the blood of rats were found not to contain oleic acid, although at least 50% of the esters of the human plasma are of the oleate type.

Glyceryl ethers

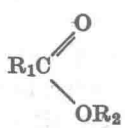
The glyceryl ethers can be represented by the formula



where R is usually palmityl, stearyl and oleyl. The R group is said to be found exclusively in the 1-position. They occur in marine animals, but only to a limited extent in land animals. Both D- and L-forms are possible but in nature the D-form is the predominant one.

Fatty alcohols and waxes

Although not found to any significant extent in land mammals the waxes do occur in aquatic animals and certain plants. The sperm whale contains considerable amounts of long-chain alcohols such as cetyl, stearyl and oleyl alcohols, esterified with long-chain acids. The waxes can be represented as carboxylic esters of the type:



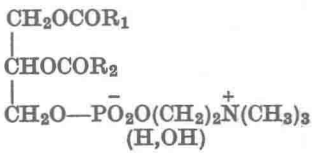
where R_1 is a long-chain fatty acid and R_2 is a long-chain alcohol. Natural leaf waxes contain alcohols such as myricyl alcohol $C_{30}H_{61}OH$ and myricyl palmitate occurs in high concentration in beeswax.

COMPLEX LIPIDS

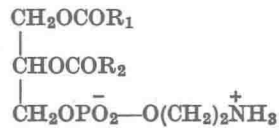
The complex lipids consist of esters which may contain phosphorus, nitrogen bases, and sugars, in addition to long-chain fatty acids. First we may consider the phospholipids containing glycerol, i.e. the phosphoglycerides.

Phosphoglycerides

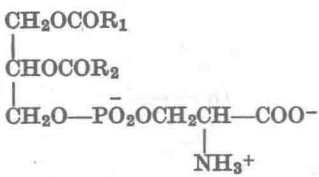
The phosphoglycerides, although apparently of little technological importance, are of widespread occurrence in nature and of great biochemical interest. These lipids have been associated with blood-clotting processes, as a source of choline in nervous tissue, as a matrix for the structure of the living cell, the transport of potassium and sodium ions and in many biological oxidations and as intermediates in the metabolism of fatty acids. Typical phosphoglycerides of importance are:



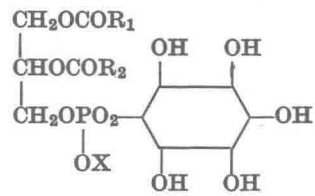
Phosphatidyl choline (α-Lecithin)



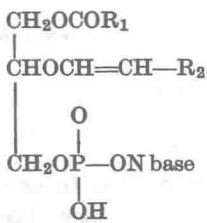
Phosphatidyl ethanolamine (α-Cephalin)



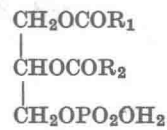
Phosphatidyl serine



Phosphatidyl inositol (X is a cation)



Plasmalogen (base, choline or ethanolamine)



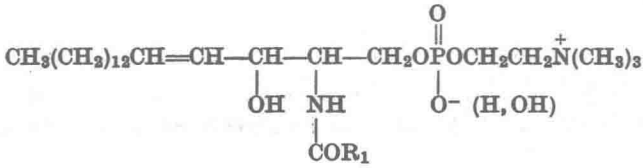
Phosphatidic acid

where R_1 and R_2 are the long-chain hydrocarbon units of the fatty acids. (With natural phosphoglycerides R_2 usually contains an unsaturated grouping.)

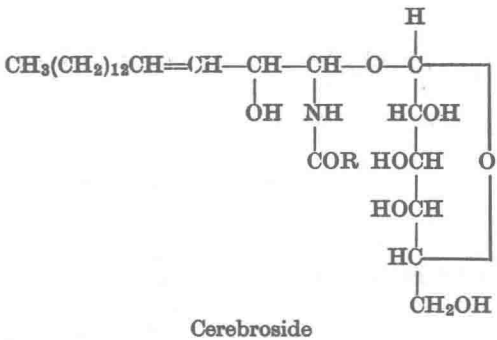
Sphingolipids

The sphingolipids are characterized by the derivation of its members from a number of long-chain hydroxylic bases; sphingosine, dihydrosphingosine, phytosphingosine and dehydrophytosphingosine. The first

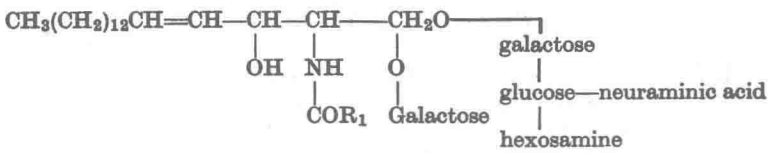
two occur in animal fats, particularly in pancreatic, brain and spinal lipids. The structure of some sphingosine-containing lipids are:



Sphingomyelin



Cerebroside



Ganglioside

Phytosphingosine and dehydrophytosphingosine appear to occur in plant sources only. Derivatives of phytosphingosine have been observed in soya-bean oil.

LIPOPROTEINS

In recent years it has become clear that lipids are also found to occur in an associated form along with proteins [1, 2, 3]. These lipoproteins occur in many systems and appear to be of considerable importance. Almost all biological membranes, cytoplasmic membranes, nuclear membranes, endoplasmic reticulum, cristae of mitochondria, lamellae of chloroplasts, myelin of nerve fibres, are composed of lipoproteins. They support the metabolic apparatus in mitochondria and the photosynthetic apparatus in chloroplasts, and are thought to be associated with enzymatic activity

in mitochondrion [4]. The 'soluble' lipoproteins are considered to be responsible for the transport of lipids in blood. All or nearly all of the lipid components in blood plasma are combined with protein [5]. The nature of the bonding between lipid and protein is still unknown, although there have been speculations about this. As an individual lipoprotein particle may contain 1000 to 2000 lipid molecules, lipid–lipid interactions are important as well as lipid–protein interactions. In the nervous system a major part of lipids and proteins occur as lipoproteins. Some of these are similar to those found in plasma, i.e. soluble in water and insoluble in organic solvents, some occur which are insoluble in water and in organic solvents and a third group occurs which are insoluble in water and soluble in certain organic solvents. The latter group has been named the proteolipids [6].

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2. Separation techniques

Many new techniques have become available in recent years for the separation of lipids from the complex mixtures in which they usually occur. Here we discuss them briefly, pointing to the particular features of the different methods.

We shall consider first the methods of separation available for the lipoproteins. As well as the general composition the fatty acid composition of the lipids in the lipoprotein complexes may significantly affect their behaviour and properties and can vary from particle to particle. This means that lipoprotein particles have first to be separated from each other and then the composition of the lipid mixtures determined according to the type of phospholipid, triglyceride and cholesterol esters present. Finally, in a complete analysis, the fatty-acid distribution is determined and the specific configuration of the fatty acid on the phospholipids or glycerol moiety deduced.

Separation of lipoproteins

One of the most characteristic properties of lipoproteins is their low density and hence the most widely used technique for separating them is by flotation using an ultracentrifuge. Determination of the sedimentation rate with varying density of the solvent can be used to differentiate the lipoproteins from other proteins of more normal density and to separate lipoproteins of differing density. The lipoproteins are themselves separated by means of their different densities; these differences reflect the densities of the individual components of the lipoproteins. There are two general procedures for separating different classes of plasma lipoproteins. In the first [1] the serum fraction is mixed with a quantity of salt solution (sometimes containing D_2O) providing a small molecule environment of a predetermined density. The lipoprotein molecules in the ultracentrifuge cell are subject to approximately the same buoyancy factor. The rate of flotation of the lipoprotein boundaries can be observed and the various components are characterized according to Svedberg's units of flotation. (The rate of flotation in a unit centrifugal field when the lipoprotein is suspended in an aqueous sodium chloride solution of density 1·063 at 26° is indicated by S_f notation.) Factors which affect the