Lipid Analysis

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Isolation, separation, identification and structural analysis of lipids

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Preface

TWENTY years ago, lipids were considered to be oily intractable substances that could be separated into simpler components only with great difficulty and they were studied by a comparatively limited number of painstaking devotees. The development of chromatographic techniques, particularly gas–liquid chromatography and thin-layer chromatography, together with advances in spectroscopy, have led to an explosive growth of interest in these compounds and have revolutionised our knowledge of the role that lipids play in the structure and function of cell membranes, as essential dietary components and in numerous biological processes. A great number of specialist journals and review articles are now published at regular intervals and in this book I have attempted to critically examine the literature and bring together in a systematic manner the best of the procedures that have been developed for separating, identifying and determining lipid classes and their component parts. Many of these methods were evaluated at the same time in my own laboratory. I hope that newcomers to the subject will find it a useful guide through the potential complexities of lipid analysis and that experts in the field will find it a valuable reference work.

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W. W. CHRISTIE

CHAPTER 1

The Structure, Chemistry and Occurrence of Lipids

A. Introduction

The term LIPID is often used loosely to denote a wide variety of natural products including fatty acids and their derivatives, steroids, terpenes, carotenoids and bile acids, which have in common a ready solubility in organic solvents such as diethyl ether, hexane, benzene, chloroform or methanol. A more specific definition is to be preferred and the term is nowadays generally restricted to fatty acids and their derivatives or metabolites. It is in this sense that the term is used in this book.

The principal lipid classes consist of fatty acid (long-chain aliphatic monocarboxylic acid) moieties linked by an ester bond to an alcohol, principally the trihydric alcohol glycerol, or by amide bonds to long-chain bases. Also, they may contain phosphoric acid, organic bases, sugars and more complex components that can be liberated by various hydrolytic procedures. Lipids may be subdivided into two broad classes—"simple", which contain one or two of these hydrolysis products per mole, and "complex", which contain three or more types of hydrolysis product per mole. The terms "neutral" and "polar" respectively are used more frequently to define these classes, but are less precise and may occasionally be ambiguous; for example, unesterified fatty acids are normally classed as neutral lipids despite the presence of the free carboxyl group.

A complete analysis of the lipids from a given source, therefore, involves separation of the lipid mixture into simpler types according to the number and nature of the various constituent parts, the identification and estimation of each of these and finally determination of the absolute amount of each lipid type. Before progressing to this, however,

a knowledge of the structure, chemistry and occurrence of the principal known lipids and their constituents is necessary.

B. The Fatty Acids

The common fatty acids of plant and animal origin contain even numbers of carbon atoms (4–24) in straight chains with a terminal carboxyl group and may be fully saturated or contain one, two or more (up to six) double bonds, which generally but not always have a *cis*-configuration. Fatty acids of animal origin are comparatively simple in structure and can be subdivided into well-defined families. Plant fatty acids, on the other hand, may be more complex and can contain a variety of other functional groups including acetylenic bonds, epoxyl, hydroxyl or keto groups and cyclopropene rings. Bacterial fatty acids usually consist of simpler saturated and monoenoic components but may also contain odd-numbered, branched-chain and cyclopropane acids. Very complex high molecular weight acids, the mycolic acids, have been found in certain bacterial species.

1. *Saturated fatty acids*

The commonest saturated fatty acids are straight-chain even-numbered acids containing 14–20 carbon atoms, although all the possible odd and even-numbered homologues with 2–30 or more carbon atoms have been found in nature. They are named systematically from the saturated hydrocarbon with the same number of carbon atoms, the final *-e* being changed to *-oic*. For example, the acid with sixteen carbon atoms and structural formula

$$CH_3.(CH_2)_{14}.COOH$$

is correctly termed hexadecanoic acid, although it also has a trivial name hallowed by common usage, i.e. *palmitic acid*. To simplify presentation and discussion of fatty acid compositions, shorthand nomenclatures also exist. In the simplest form, fatty acids are designated solely by the number of carbon atoms they possess, e.g. palmitic acid is a C_{16} acid. This compound can be defined more accurately, however, by listing both the number of carbon atoms in the acid and also the

number of double bonds, separating the two figures by a colon, i.e. taking the above example—16:0. Table 1.1 contains a list of common saturated fatty acids together with their trivial and systematic names and shorthand designations.

TABLE 1.1. SATURATED ACIDS OF GENERAL FORMULA $CH_3.(CH_2)_n.COOH$

Systematic name	Trivial name	Shorthand designation
ethanoic	acetic	2:0
propanoic	propionic	3:0
butanoic	butyric	4:0
pentanoic	valeric	5:0
hexanoic	caproic	6:0
heptanoic	enanthic	7:0
octanoic	caprylic	8:0
nonanoic	pelargonic	9:0
decanoic	capric	10:0
hendecanoic	—	11:0
dodecanoic	lauric	12:0
tridecanoic	—	13:0
tetradecanoic	myristic	14:0
pentadecanoic	—	15:0
hexadecanoic	palmitic	16:0
heptadecanoic	margaric	17:0
octadecanoic	stearic	18:0
nonadecanoic	—	19:0
eicosanoic	arachidic	20:0
heneicosanoic	—	21:0
docosanoic	behenic	22:0
tetracosanoic	lignoceric	24:0

Acetic acid is rarely found in association with higher molecular weight fatty acids in esterified form, although it has been found esterified to glycerol and to hydroxy-fatty acids in some seed oils. C_4 to C_{12} acids are found mainly in milk fats, although the C_{10} and C_{12} acids have also been found in quantity in certain seed oils. *Myristic acid* (14:0) is a minor component of most animal lipids, but is present in major amounts in seed oils of the family Myristicaceae. Palmitic acid is probably the commonest saturated fatty acid and is found in virtually all animal and plant fats and oils. *Stearic acid* (18:0) is also relatively