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R.H. BURDON and P.H. van KNIPPENBERG
Editors

techniques of lipidology

Isolation, analysis and identification of lipids

2nd revised edition

MORRIS KATES

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ISOLATION, ANALYSIS AND IDENTIFICATION OF LIPIDS

2nd revised edition

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LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

Volume 3, part 2

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Preface to the Second Edition

Since the appearance of the first edition of this book at the end of 1972, the number of publications dealing with the isolation, analysis and identification of lipids has increased enormously. Modifications and improvements of established analytical techniques, and new analytical procedures have been introduced, such as increasingly sensitive NMR, IR, and mass spectrometric methods, and high-performance liquid chromatography. New lipid components have been discovered and the range of previously known lipid classes has been greatly extended. Several new books dealing with lipids and lipidology have appeared, for example, *Lipid Biochemistry* (Gurr and James 1980), *Lipid Biochemical Preparations* (Bergelson 1980), *Lipid Analysis* (Christie 1982), *Lipids in Plants and Microbes* (Harwood and Russell 1984) and *Biochemistry of Natural C–P Compounds* (Hori et al. 1984).

As a result, the present edition has become somewhat outdated and in need of revision. I have tried in this second edition not only to update procedures and instrumentation but also to enlarge the theoretical basis of the book when appropriate. For example, chemical structures of new lipid classes and components have been added and chemical reactions underlying analytical procedures, as well as formulae for calculation of analytical results, have been inserted. Procedures and techniques that are no longer useful or have been superseded or improved have been deleted or replaced by newer procedures. However, the overall format and organization has essentially not been altered. The index has been greatly expanded and

the cross-referencing has been increased.

It is hoped that this revised edition will be more useful to a wider group of researchers, as well as to the professional lipidologist, than the previous one.

Preface to the First Edition

Lipidology, the study of the chemistry and function of lipid or fatty material, has until recently appealed to a relatively small number of chemists and biochemists. Perhaps this has been due to the apparently intractable nature of the 'smears', 'greases' and 'oils' which are usually encountered in this field; or perhaps the question of the precise role of these heterogeneous substances in living systems appeared to be insoluble or not worth pursuing.

This attitude towards Lipidology has now completely changed as a result of several new developments. One of the most important was the development of chromatographic techniques — paper, gas–liquid, and thin-layer chromatography — and their application to the analysis, separation, and isolation of lipid components in natural mixtures. Equally important was the development of synthetic methods which made available pure lipids of known structure (Baer 1965). Another was the application of electron microscopic techniques to cytology and the realization that much of the biosynthetic and metabolic function of the cell occurred on or in association with membranes. Finally, it has become clear that cellular lipids are largely present as membrane constituents and that the function and properties of membranes are to a great extent determined by the chemical structure of these lipid components and their orientation in the membrane structure.

It was in the mid-1950's that interest in the study of lipids began to increase greatly and to spread among biochemists, biologists, plant physiologists, microbiologists and biophysicists. New journals

devoted entirely to lipids began to appear, joining the two previous journals of this type, the *Journal of the American Oil Chemists' Society* and *Fette, Seifen und Anstrichmittel*. At the present time there are at least four additional publications devoted entirely to lipids: the *Journal of Lipid Research* (founded in 1959), the specialized section on Lipids in *Biochimica et Biophysica Acta* (begun in 1963), *Lipids* (begun in 1966) and *Chemistry and Physics of Lipids* (founded in 1966). Furthermore, a serial publication *Advances in Lipid Research* was started in 1963 to review developments in specialized areas of Lipidology on a comprehensive and critical level, a much-needed addition to the older series *Progress in Chemistry of Fats and other Lipids*. Of course, many research publications on lipids will still be found in the established biochemical journals (*Journal of Biological Chemistry, Biochemical Journal, Biochemistry*, etc.), and also recently, in such biological journals as *Journal of Bacteriology*.

My purpose in writing this book has been two-fold. The first was to collect into one volume the basic knowledge and experimental procedures necessary for the study of the chemistry and metabolism of lipids. The second was a purely selfish one, namely to have all the procedures and methods which we use in our work in one easily accessible source. In approaching the writing of this book, it soon became clear that it would be impossible to include all of the most recent developments in lipidological techniques, at least in any great detail, and that a considerable degree of selection would have to be exercised. The approach finally adopted was to include those methods and procedures, in their most recently modified form, that have proved to be most reliable and useful over the years, according to my own experience and that of colleagues.

The level of presentation and the scope of the material had also to be considered. It was decided that for optimal usage, the book should be aimed at the overall level of the advanced graduate student; however, it was felt that the book should be of sufficient calibre to enable the professional chemist, biochemist, biologist or other scientist to work in the lipid field without extensive prior

experience. The professional lipidologist, it is hoped, will also find it convenient to have many of his standard procedures collected in one volume.

As to the range of material presented, it was decided to restrict the coverage to information necessary for the qualitative and quantitative analysis of cellular or tissue lipids, and for identification and structural determination of lipid classes or individual components. It is hoped that the biochemist or biologist wishing to extract, analyse, and identify cellular or tissue lipids will find most of the necessary procedures and data at hand in this book.

Acknowledgements

Second Edition

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First Edition

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List of abbreviations

ACP	Acyl carrier protein.
t-BDMS	*tert.*-Butyldimethylsilyl ethers.
CDP	Cytidine diphosphate.
CTP	Cytidine triphosphate.
DEAE	Diethylaminoethyl.
DMA	Dimethyl acetal.
DNP	2,4-Dinitrophenyl.
DNPH	Dinitrophenylhydrazine
EDTA	Ethylenediamine tetraacetic acid.
ESR	Electron spin resonance.
GC–MS	Gas chromatography–mass spectrometry.
GLC	Gas–liquid chromatography.
HPLC	High-performance liquid chromatography.
IR	Infrared.
LC	Liquid chromatography.
NMR	Nuclear magnetic resonance.
PA	Phosphatidic acid.
PC	Phosphatidyl choline.
PE	Phosphatidyl ethanolamine.
PG	Phosphatidyl glycerol.
di-PG	Diphosphatidyl glycerol (cardiolipin).
PI	Phosphatidyl inositol.
PS	Phosphatidyl serine.
SPH	Sphingomyelin.
TEAE	Triethylaminoethyl.
TLC	Thin-layer chromatography.
TMS	Trimethylsilyl.
Tris	Tris-(hydroxymethyl)-aminomethane.

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