Plant Lipids

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Plant Lipids

Biology, Utilisation and Manipulation

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Preface

It is now over 30 years since publication of the seminal work by Hitchcock and Nichols on Plant Lipid Biochemistry (Academic Press, 1971). It is also over 15 years since the most recent updating of the comprehensive treatise on plant lipids edited by Stumpf and Conn (Lipids, Vol. 9 in the *Biochemistry of Plants* series, published in 1976 by Academic Press and revised in 1987). Since the publication of these two books, the field of plant lipids has changed almost beyond recognition. New research tools have revealed many surprising aspects of the dynamic nature of lipids and their participation in processes such as recognition, intra- and intercellular signalling, deterrence and defence against pathogens, membrane trafficking and protein function. This is in addition to new information on the more established roles of plant lipids as structural components of membranes and as long-term storage products. Plant lipids are also increasingly being seen as sources of a new generation of environmentally friendly, biodegradable and renewable industrial products, including biopolymers and high grade lubricants.

Over the past three decades, the proceedings of the biennial International Plant Lipid Congresses and other meetings have been published as collections of brief papers. There have also been monographs derived from meetings that concentrated on specific aspects of plant lipids, such as their biotechnological manipulation. However, no broad overview of plant lipids has been available since the two much earlier (and more biochemically-focused) works mentioned in the previous paragraph. In the present volume, researchers from major international laboratories have been brought together to provide reviews of progress in plant lipid research and its many applications. The intention is to link the various disciplines that are related to plant lipid research, in order to provide an interesting and wide-ranging perspective on this fast-moving field. There is a deliberate measure of overlap in some of the chapters where this sheds additional light on a topic from a different viewpoint or in a different context.

Chapter 1 provides a historical perspective on the study of plant lipids from its inception as a branch of alchemy in the seventeenth century to the interdisciplinary research of the post-genomic era in the twenty-first century. As new techniques become available, plant lipid research has advanced in both new and more traditional ways. Hence, the powerful tools of modern molecular genetics have contributed immensely to a far deeper understanding of lipid biochemistry and its underlying enzymology, even to the extent that some well established metabolic pathways are now in the process of being revised.

The next three chapters discuss aspects of the formation, modification and utilisation of fatty acids. Chapter 2 deals with the biosynthesis *de novo* of fatty acids from their earliest carbon precursors, through their assembly on the various types of fatty acid synthase complexes in plants, to their final release by thioesterases. The chapter also discusses recent ideas about the metabolic regulation of fatty acid formation. Chapter 3 considers the various modification reactions that fatty acids undergo after their synthesis *de novo*, and the ways in which these processes are being manipulated to create novel fatty acids that may be of interest to the biotechnology industry. Chapter 4 discusses the vast range of naturally occurring fatty acids, their glycerol esters and their many actual or potential uses as non-edible products. This chapter also provides an overview of the physical and chemical characteristics of acyl lipids.

The next two chapters deal with the major classes of macromolecular structures formed by plant lipids, namely bilayer membranes and storage bodies. Chapter 5 examines the structure, localisation and biosynthesis of the membrane glyco- and phospholipids and considers the roles of lipases in their turnover. This is followed by a discussion of the physiological role of membrane lipids in processes such as thermal tolerance and photosynthesis. Chapter 6 is a comprehensive account of the metabolism of storage lipids, including the various biosynthetic routes to triacylglycerol formation, lipid-body assembly and their subsequent degradation by lipolysis and β-oxidation. The chapter also covers physiological aspects, including environmental effects on storage lipid formation and their possible transport within plants.

Chapters 7 and 8 are concerned in different ways with some of the other types of macromolecular lipid assemblies in plants. Chapter 7 examines the roles of the various lipid-associated proteins in plants and compares these with analogous proteins in other organisms, including animals and microbes. These diverse proteins suggest new roles for some plant lipid assemblies that include membrane trafficking and dynamic interactions in physiological events such as stress responses and senescence. Chapter 8 focuses on that key plant lipid structure, the cuticle. The chapter covers the elaboration of fatty acids to form very long chain acyl derivatives and their assembly into waxes. The spatial context of this process is discussed, as is the physical nature of the various forms of crystalline wax that may be deposited under different conditions.

The final three chapters are concerned with three diverse classes of plant lipids that have numerous functions but are linked in their contributions to various aspects of signalling, either as part of normal development or during stress responses, both biotic and abiotic. Chapter 9 deals with the wide range of inositol-containing lipids, their derivatives and the proteins with which they interact. Comparisons with non-plant systems reveal both similarities and differences and highlight the many key roles of phosphoinositides in many fundamental signalling processes in plants. Chapter 10 examines the diverse class of oxygenated fatty acid derivatives, the oxylipins, which are now known to play a crucial role in the response of plants to

pathogens and to more general wounding by herbivores. In particular, the formation and signalling roles of jasmonates and their interactions with other signalling pathways are described. Chapter 11 covers a vast range of lipidic compounds, namely the prenyllipids. Key members of this group are described, including quinones, carotenoids, sterols and terpenoids. The utilisation and manipulation of important prenyllipids, like carotenoids and taxol, are also discussed.

This book introduces the reader to the various aspects of plant lipids, summarises current developments and provides a resource for further study. To facilitate the latter aim, the authors have provided extensive lists of references, totalling well over two thousand non-redundant citations. It is hoped that this book will inform and stimulate the reader, as well as demonstrating the dynamic nature of plant lipid research. We also highlight some of the exciting opportunities for the application of this research in medicine and agriculture. Finally, we would point to the many new avenues that are now opening to young investigators who, hopefully, may be encouraged to pursue a career in furthering our knowledge of plant lipid science.

Denis J. Murphy

1 The study and utilisation of plant lipids: from margarine to lipid rafts

Denis J. Murphy

1.1 Introduction

This chapter will take a retrospective look at the study and utilisation of plant lipids, especially acyl lipids, and set current developments in a historical context. It is meant to be an outline description of the field, rather than a comprehensive history of the topic – perhaps the latter task is something that one of our readers might be inspired to undertake at some time in the future.

1.2 Early studies of plant lipids

Plant lipids are all around us, both in our environment and in our food. They are the second most important source of edible calories in the human diet (after carbohydrates). Indeed, for millions of years, they have also been a key source of those essential fatty acids (EFAs) that are an obligatory component of the diet of all mammals – ever since our distant animal ancestors lost the ability to introduce double bonds beyond the Δ_9 position in long-chain fatty acids. Since the dawn of agriculture, over 11 000 years ago, certain plant species have been cultivated specifically for their lipid composition. The earliest olive plantations have been dated to more than 9 millennia before the present day and maize may have been domesticated in Mesoamerica as early as 10 millennia ago. Globally, there are now just 15 major crops that supply most of the human diet and five of these are high-oil crops, namely, soybeans, oil palm, maize, peanuts and coconut (Harlan, 1992). Plant oils have also been utilised by human societies for a host of non-edible applications, ranging from fuels to lighting and from cosmetics to lubricants. It was only the easy availability of plentiful, inexpensive, fossil-derived hydrocarbons (petrochemicals) in the late nineteenth century that displaced plant oils from many of their more prominent non-edible applications (oleochemicals). With the end of the era of cheap fossil hydrocarbons now in sight, probably within the present century (Murphy, 2004), plant lipids will once again be required in order to produce many of the multitudes of economically important non-food commodities that we depend upon so much.

Despite their impressive pedigree as important sources of food and raw materials for human societies, the systematic study of plant or animal lipids did not begin until after the inception of the modern scientific revolution in the seventeenth century. One of the earliest landmarks in lipidology was the publication in 1666 of the Hippocrates Chymicus by the German alchemist, Otto Tachenius. In this book, Tachenius first suggested that fats contain an acidic substance – what we now call fatty acids. He was also the first person to give a distinct definition of salt when he wrote that 'all salts are composed of two parts, of acid and alkali'. He further added that soap was the salt of an oily acid. Tachenius' statements were not accepted by the community of the time, and it was not until the French chemist Chevreul rediscovered the idea in 1816 through his laboratory work that Tachenius' definition of a salt was finally accepted (Partington, 1989).

Relatively little work of note was done on plant lipids until the nineteenth century, when many important contributions were made by French and Germanic chemists in particular. An early landmark occurred at the beginning of the nineteenth century when the Swiss chemist, Nicolas-Théodore de Saussure, demonstrated that linseed oil could condense with oxygen: this was an early hint of the existence of double bonds (de Saussure, 1804). In 1816, the renowned French chemist, Magendie, found that dogs that were fed on a diet in which the only lipid component was olive oil did not live for more than a month. Although olive oil contains about 7% linoleic acid, the high oleic/linoleic ratio would have led to marginal EFA deficiency in these animals. This result, although not appreciated at the time, flagged up the nutritional importance of some classes of polyunsaturated fatty acids (which are lacking in olive oil) and of antioxidant vitamins, including the A and E complexes.

The compositions of the major plant oils were gradually uncovered over the next few decades: with the suggestion that oleic acid and 'margaric acid' (a mixture of palmitic and stearic acids) were present as a mixture in vegetable oils (Pelouze and Boudet, 1838); the discovery of myristic acid in seeds of the Myristicaceae (Playfair, 1841); the isolation of lauric acid in the seeds of laurel, *Laurus nobilis* (Marsson, 1842); the preparation of linoleic acid from linseed (*Linum usitasissimum*) oil (Sacc, 1844) – although it was nearly a century before its structure was elucidated by Hilditch in 1939; the isolation of first known hydroxy fatty acid, ricinoleic acid (18-1-OH), from castor oil (Saalmüller, 1848); and finally, the description (Darby, 1849) and purification (Websky, 1853) of erucic acid from rapeseed oil. A link with the principal membrane lipids of animals occurred when phospholipids, often called lecithins at the time, were also shown to be present in plant seeds (Töpler, 1861), while lipases were first demonstrated in plant seeds by Muntz (1871). The chemical structure of oleic acid, including the position of the double bond, was first described by Edmed (1898) following an elegant series of oxidation steps. With this development, the stage was set for the systematic elucidation of the structures of each of the major plant fatty acids, although this task would take several more decades before it was finally achieved.

Meanwhile, the first major commercially relevant technological innovation for plant lipids came in 1869 when a French chemist called Hippolyte Mège Mouriès produced what we now know as margarine. Even in those days, plant lipid researchers had to respond to the needs of industry and government and the work of Mège Mouriès followed a call by the French emperor, Napoleon III, for an alternative to butter. Although it has been claimed that this was done to enhance the nutrition of the working classes, another (doubtless more pragmatic) motive was to provide a cheaper and more versatile source of food for the French army, which was then preparing for a conflict with the emergent state of Prussia for domination of continental Europe. As a raw material for the new product, Mège Mouriès selected a particular solid fatty acid fraction that was called margaric acid. This name derives from the lustrous pearly-white drops of the crystalline form of margaric acid that are reminiscent of pearls and which are called margarites in Greek (this is also the derivation of the name Margaret).

The earliest forms of margarine were mixtures of animal and plant fats, but the product was yet to be a great commercial success. Two technical advances tipped the balance towards plant fats in margarine and allowed it to be an effective competitor with butter. First, improved refining methods allowed the purification of a greater variety of liquid oils and solid vegetable fats that could be blended to make good spreadable margarine. Second, the process of hydrogenation, which was invented in 1901 by English chemist William Normann, allowed the large scale conversion of relatively cheap plant oils into solid fats. Not only did the hydrogenation process produce a good, inexpensive butter substitute, it also significantly reduced the amount of oxidation-prone polyunsaturates in the solid margarine, which greatly extended its shelf life and, therefore, its utility for consumers.

Like many start-up researcher/entrepreneurs of the present day, Mège Mouriès soon sold on the rights to his invention to a larger, more established company and, in 1871, the technology was acquired by the Dutch firm, Jurgens, which is now part of the Unilever group. Today, Unilever is one of the largest multinational enterprises that use plant lipid products, including margarines and detergents, as a core part of their business. Unilever scientists have also made key contributions to the study of plant lipids. For example, it was at the Unilever research labs in the United Kingdom that some of the most significant advances in lipid analysis were made in the 1960s and 1970s, as described in later sections.

The dawn of the twentieth century was appropriately marked by the demonstration of his eponymous engine by Rudolf Diesel at the International Exposition, held in Paris in 1900. The first diesel engine was powered by peanut oil, so it can be said that plant lipids played an important role in ushering in the age of the automobile (Nitske, 1965). However, plant-derived oils were almost immediately abandoned in favour of a petroleum fraction that is slightly heavier than gasoline. This fuel was named after the inventor of the engine and has been called diesel ever since. Ironically, the past decade has witnessed a partial return to plant-derived diesel fuels, normally based on methyl ester derivatives of seed oils from crops like rapeseed and

sunflower. These so-called 'biodiesel' fuels have improved environmental impacts compared to regular diesel, especially in sensitive areas like inland waterways and city centres. Despite their undoubted 'green' credentials, however, biodiesel fuels remain economically questionable as petrodiesel substitutes and their use is likely to be more or less completely dependent on support from government subsidies for the foreseeable future (Murphy, 1998, 2004).

One of the earliest examples of an international honour for a plant lipid researcher came in 1915 when R.M. Willstätter was awarded the Nobel Prize for chemistry 'for his research on plant pigments, especially chlorophyll'. Willstätter had worked on the technique of chromatography late in the nineteenth century and had undertaken some particularly heroic extractions, such as the isolation of chlorophyll from several hundred kilograms of dried stinging nettles in 1905 (Willstätter, 1973).

By the early twentieth century, the results of plant lipid science were being applied widely in both industry and medicine. For example, the use of interesterification for the production of oleochemicals was patented in the United Kingdom by van Loon in 1924 (UK patent # 249 916), while in 1924, the essential role of vitamin E was described and the name was first suggested (Sure, 1924). This was followed a few years later by the discovery of the essential role of long-chain polyunsaturated fatty acids in the diet (Burr *et al.*, 1930). A further Nobel Prize followed in 1930 when R. Kuhn was honoured for his work on carotenoids and vitamins.

Plant lipid research was now about to enter the next stage of its development, which was characterised in particular by several key advances in analytical methodology and instrumentation.

1.3 The chemistry era – and the definition of the term 'lipid'

Most of the really important developments in advancing the chemical study of plant lipids came after World War II. However, there were a few discoveries that can be regarded as precursors for these later successes, most of which relate to the discovery and application of various forms of chromatography. Investigators like Willstätter in Germany had been experimenting with different techniques for the separation of plant lipids since the late nineteenth century, but the first public description of the chromatographic technique was given by Mikhail Tswett at a meeting in Warsaw in 1903. Tswett coined the term 'chromatography' and used the new method for the separation of plant pigments on a chalk column, although it took several more decades before the technique was taken further. In 1938, a Soviet group described the use of thin-layer chromatography (TLC) on microscope slides coated with aluminium oxide (Izmailov *et al.*, 1938). This group, based in Kharkov in the Ukraine, originally developed this early version of TLC to separate plant chemicals of potential pharmaceutical interest, the so-called galenic extracts. A further landmark came in 1940 when T.P. Hilditch, in the United Kingdom, published the seminal and much reprinted volume entitled *The chemical constitution of natural fats* (Hilditch, 1940).

It was in the 1947 edition of this book that Hilditch proposed that the term 'lipid' should be used. As Hilditch stated:

unanimity has not yet been reached in the terminology to be adopted in classifying the various types of naturally occurring compounds in which fatty acids are present . . . even a collective title for the whole group is not completely settled.

Prior to this, there had been several competing terms used in the English literature, including 'lipoids', which tended to be used by British investigators, and 'lipins', which was favoured by Americans. An interesting hangover from this terminology is the current use of the term 'oxylipin' for oxygenated lipid derivatives, as employed in Chapter 10 of the present volume. The term 'lipid' had originally been proposed by Sperry (1926), but was not widely adopted until Hilditch's suggestion in 1947.

Lipids were originally defined according to chemical criteria, namely, as fatty acids and their derivatives. However, it has become more common to define lipids on the basis of their physical properties, namely, as oily, fatty or waxy organic compounds, which while insoluble in water are readily soluble in organic solvents. Obviously, exceptions can be found to the latter definition, e.g. it would not apply to some of the more polar monoacylglycerols or fatty acid salts. Even no less an authority than Bill Christie admits that, 'there is no widely-accepted definition' of a lipid (Christie, 2003). Historically, the term 'fats' was used for naturally occurring 'triglycerides', whether solid or liquid (these are now correctly called triacylglycerols – although, alas, this seems to have escaped the attention of some in the field). Lipids can also encompass a host of other naturally occurring non-polar compounds that do not contain acyl groups. These include a host of prenyl derivatives including carotenoids, tocopherols, terpenes and quinones as well as phytylated pyrrole derivatives, such as the chlorophylls, and numerous families of fatty acid derivatives, some of them relatively polar, including eicosanoids, oxylipins and volatiles like hexenal and pentenone (see Chapter 4 for more on lipid definitions).

Despite the international conflicts of the 1940s, there were some notable advances in lipid analysis during this decade. Martin and Synge (1941) first described partition chromatography at a meeting of the Biochemical Society in the then war-torn city of London. Both investigators subsequently shared the 1952 Nobel Prize for Chemistry 'for the invention of partition chromatography'. Many lipid analyses at this stage employed large amounts of starting material, as described above in the case of Willstätter and his stinging nettles. This was necessary, either because the lipids of interest were such minor components and/or because the relatively crude detection systems that were then available required large quantities of purified lipid. Therefore, the development of an initial purification step involving high-capacity column chromatography was of great use. Early columns used alumina and could separate non-saponifiable lipid mixtures into several fractions (Swain, 1948), although silicic acid columns were soon developed and used to separate relatively complex mixtures of neutral lipids and phospholipids (Borgström, 1952). Reversed-phase partition chromatography on Kieselguhr was first used to separate C12–C18 fatty acids by

Howard and Martin (1950), followed shortly thereafter by the first application of TLC to lipids – in this case, terpenes from citrus juices, which were studied by Kirchner's group at the USDA Agriculture, Fruit and Vegetable Laboratory in Southern California (Kirchner, 1951). TLC was developed further by Stahl and colleagues at Saarbrücken in Germany in the mid- to late 1950s and by the end of the decade, he and other workers, such as Weicker (1959) in Germany and Mangold (1959) at the Hormel Institute in Austin, Minnesota, had developed silica-based TLC into a highly precise and accurate technique that could be used for both preparative and analytical separations. The utility of TLC was extended further by the usage of impregnated silica or other stationary phases. For plant lipid researchers, one of the most useful methods is argentation-TLC, whereby silver nitrate is added to the silica. This allows for the separation of long-chain fatty acyl groups on the basis of their unsaturation, as well as chain length, and is especially valuable for analysis of plant acyl lipids because of their high degree of unsaturation.

Meanwhile, in the early 1950s, A.J.P. Martin, who had jointly invented partition chromatography a decade earlier, teamed up with another young chemist, A.T. James, in London. This duo presented a paper announcing the extension of partition chromatography to include a gas as the mobile phase at a Biochemical Society meeting in 1950. This marked the birth of gas–liquid chromatography or GLC, and the landmark paper describing the technique appeared 18 months later (James and Martin, 1952). Part of the impetus for developing GLC came from a colleague of James and Martin, who was keen to find an alternative to paper chromatography for the effective resolution of fatty acid mixtures. Since then, gas chromatography (GC), as it became known, has developed rapidly, particularly during the 1960s, to provide both a preparative and an analytical tool for lipid, and especially fatty acid, analysis. The technique has now been applied in almost every area of analytical and biochemical research. Since the mid-1990s, hybrid techniques have been developed that have added a further dimension to GC analyses. Probably the most effective of these is the use a mass spectrometer as a detector rather than straightforward thermal conductivity or flame ionisation detection systems. By employing a mass spectrometer in tandem with GC (called GC–MS), one can effectively do a two-dimensional separation and analysis of a lipid mixture. A further refinement is to add a second mass spectrometer detector in series (called GC–MS–MS), which enables the simultaneous separation and identification, based on comparison with existing libraries of spectra stored on the computer, of thousands of compounds in a mixture. This approach is the basis of 'metabolomics', as discussed in Section 1.6.

Numerous additional physical and chemical techniques were applied to the analysis of plant lipids from the late 1950s and beyond. Examples include the many forms of spectroscopy, such as ultraviolet, infrared, Raman and nuclear magnetic resonance; X-ray crystallography; hydrogenation; oxidation; and the various staining reagents that ranged from iodine vapour and flourescein sprays to charring with concentrated mineral acids. Not all of these methods can be described here, but their use in the 1960s and 1970s has been comprehensively reviewed elsewhere

(Hitchcock and Nichols, 1971; Gurr and James, 1971–1991; Gunstone, 1976, 1976). Other techniques used for plant lipid analysis include the various forms of calorimetry, such as differential scanning calorimetry and differential thermal analysis that have been especially valuable in the study of the phase behaviour of lipids, both in pure form and as mixtures *in situ*. There are several more recent manuals relating to the chemical analysis of lipids, which describe some of the contemporary techniques that are available (Christie, 1992–2003, 2003; Gunstone *et al.*, 1994; Grob and Barry, 1995; Baugh, 1997; McDonald and Mossoba, 1997; Hamilton, 1998).

1.4 The biochemistry era

Prior to the 1950s, most studies of plant lipids tended to focus on the chemical analysis of the lipids themselves, i.e. their extraction, separation, purification and the description of their structures. One example of such work is the first isolation of the key intermediate, phosphatidic acid (1,2-diacyl-*sn*-glycerol-3-phosphate), from cabbage leaves by Chibnall and Channon (1927). Unlike microbial researchers, plant investigators at this stage did not yet have access to powerful genetic tools, which would assist in the elucidation of the complexities of lipid metabolism *in vivo* (but see the next section for details of an exception to this, namely rapeseed/canola studies in the 1950s–1970s). This meant that much of the early work on lipid biochemistry relied greatly on inferences from microbial and animal systems. This situation began to change in the early 1950s when several technical advances encouraged a new generation of researchers to tackle the formidable challenges posed by lipid metabolism in plants. As described above, the progress in analytical techniques greatly facilitated the separation and identification of acyl lipids in particular. In addition, one of the more useful spin-offs from the nuclear weapons programmes, which were initiated in the 1940s, was the availability of radioactive isotopes, such as ^{14}C, ^{3}H, ^{32}P and ^{35}S. This opened the door to the synthesis of radiolabelled tracers like [^{14}C]acetate or [^{14}C]CO_2. Such tracers were especially powerful tools as they could be used to follow the pathways of metabolites in either *in vitro*, cell-free systems or with *in vivo* systems that might consist of a whole organism or a detached organ or tissue such as a leaf disc.

The foundations of plant lipid biochemistry, as we now know it, were laid in the 1950s and 1960s when a relatively small number of groups began the detailed description of the main processes of fatty acid and acyl lipid metabolism. This period saw the emergence of a series of plant lipid biochemistry labs in Europe and North America, with the University of California (UC) making a particularly prominent contribution at its various campuses. These investigators included Beevers at UC Santa Cruz, Mudd at UC Riverside and, most prominently, Stumpf at UC Berkeley and then UC Davis. In 1958, the former university agricultural station at Davis, a small town about an hour's drive inland from the San Francisco Bay Area, was established as an independent campus of the UC system. It was on this new campus

that Paul Stumpf (together with Eric Conn and Lloyd Ingraham) established the Department of Biochemistry and Biophysics and set up what became the world's most influential plant lipid biochemistry lab over the next few decades. As early as 1952, Stumpf's group was using radiolabelled compounds to investigate fatty acid biosynthesis in slices from peanut cotyledons and other plant tissues. Among other achievements, they found that acetate was the most effective exogenous fatty acid precursor (Newcombe and Stumpf, 1952). In 1955, the structure of coenzyme A (CoA) was elucidated (Baddiley, 1955), and by 1962 Stumpf's group had discovered the fatty acyl thiokinase (now called acyl-CoA synthetase) (Barron and Stumpf, 1962). Throughout the 1960s, Stumpf's group went on to elucidate the mechanism of fatty acid biosynthesis in plants with their description of thioesterases (1965), acetyl CoA carboxylases (1966) and the purification of acyl-binding protein, or ACP (1968). By the end of the 1960s, the outline and many of the details of plant fatty acid biosynthesis *de novo* were elucidated.

The various modification reactions of plant fatty acids, including desaturation and hydroxylation, were also characterised in the 1960s. The aerobic pathway of fatty acid desaturation, originally described for yeast and involving an acyl-ACP substrate (Bloomfield and Bloch, 1960) was soon shown to also apply to plants (Mudd and Stumpf, 1961; James, 1963; Stumpf and James, 1963). A.T. James (who had developed the first GC system with Martin in the early 1950s – see Section 1.2) went on to establish a particularly influential lipid biochemistry group at the Unilever Research Labs in Colworth, United Kingdom, where he was joined by workers such as Gurr, Nichols, Morris and Hitchcock. During the 1960s–1970s, this team made impressive contributions in the field of plant lipids as well as doing important work with microbial and animal systems. Although desaturation from stearate to oleate was relatively easily demonstrated, it was more challenging to elucidate the higher desaturations required to produce polyunsaturates. One problem was that whereas isolated chloroplasts could synthesise fatty acids as far as oleate, further desaturations required the presence of additional cytosolic fractions (i.e. microsomes). It was the Unilever group of Nichols, Gurr and colleagues who eventually showed that these higher desaturations occurred on oxygen ester substrates, and especially on phosphatidylcholine (Nichols *et al.*, 1967; Gurr *et al.*, 1969). This group, together with the labs of both Gunstone and Stumpf, also laid the foundation for elucidation of other fatty acids modifications in plants, including hydroxylation, conjugated desaturation and *trans* desaturation (reviewed in Hitchcock and Nichols, 1971).

The assembly of acyl groups onto glycerol backbones to produce acylglycerols was first characterised in *E. coli* and is known as the Kennedy pathway. Stumpf, Kates and co-workers showed that the same mechanism of stepwise acylation applied to higher plants (Barron and Stumpf, 1962; Sastry and Kates, 1966). It was already known that, unlike animals, most membranes in plant photosynthetic tissues were composed primarily of glycolipids – principally mono- and di-galactolipids. Galactolipids had been originally discovered in wheat flour by Carter *et al.* (1956).

However, it still came as a surprise when, in 1959, Benson at the Scripps Institute of Oceanography near San Diego, California, discovered an entirely new type of acyl lipid that contained sulfur, namely, sulfoquinovosyldiacylglycerol (Benson, 1959, 1963). Originally discovered in the microalga Chlorella, these sulfolipids were subsequently shown to be present in the thylakoid membranes of all lower and higher plants. The assembly of galactolipids, from UDP-galactose and diacylglycerol precursors, was demonstrated using isolated chloroplasts and [^{14}C]galactose in a series of papers by Mudd and colleagues (Ongun and Mudd, 1968; reviewed in Leech and Murphy, 1976). Benson (1963) suggested that sulfolipids were synthesised by an analogous mechanism involving a nucleoside diphosphate sulfoquinovose precursor that had already been detected in extracts of Chlorella cells. The only prominent phospholipid in plant photosynthetic membranes is phosphatidylglycerol and early studies of its synthesis were undertaken by the groups of Benson in the United States of America, Kates in Canada and Douce in France (Benson and Maruo, 1958; Benson and Miyano, 1961; Douce *et al.*, 1966; Sastry and Kates, 1966). The characterisation of the other major phospholipids, which are mostly extraplastidial in higher plants, was started in the 1960s in the labs of Benson, Kates and Nichols (reviewed in Hitchcock and Nichols, 1971). During the 1970s, workers such as Galliard also made key contributions in the early characterisation of the various forms of lipid oxidation reactions that are now known to play such important roles in processes ranging from flavour generation to hormonal signalling in plants.

Regarding lipid catabolism, Stumpf's lab once again played an important role in showing that β-oxidation of fatty acids occurred in plants (Stumpf and Barber, 1956). The groups of both Beevers and Stumpf then showed that β-oxidation takes place in a newly discovered organelle, the glyoxysome (Cooper and Beevers, 1969; Hutton and Stumpf, 1969). Over the next two decades, Beevers' group went on to characterise the details of plant β-oxidation and other aspects of glyoxysomal metabolism, including the glyoxylate cycle and generation of sucrose from catabolised fatty acids. Plant lipases acting under non-physiological (acid) conditions had been discovered previously, but the first neutral lipase was described by Yamada (1957). This enzyme was shown to be located on lipid bodies in germinating seedlings (Ching, 1968).

Despite all of these advances in the 1960s, plant lipid biochemistry at this stage still remained something of a Cinderella discipline, compared to the much larger and better-resourced animal and microbial groups. The emergence of plant lipids as an area of research with its own distinctive identity and unique personalities came about largely thanks to an initiative by a group of British workers in the early 1970s. The initial landmark was the publication in 1971 of the first textbook for plant researchers, *Plant Lipid Biochemistry* (Hitchcock and Nichols, 1971). This book has been an invaluable resource for many of us and my own much-thumbed copy still occupies a privileged and readily accessible place right above my office desk. However, in the early 1970s, there was still no real regular forum for plant

lipid researchers to present and discuss their work, except as a relatively marginal part of meetings dominated by animal and microbial investigators. As Galliard and Mercer stated in 1975:

Those engaged in research on plant lipids have suffered in the past at biochemical meetings in which colleagues working with animal or microbial systems have dominated the proceedings. One of us has resorted to using only the term Solanum tuberosum when referring to the humble potato in the hope that the non-phytochemical majority might think that this was the name of a micro-organism and thus be acceptable to the biochemical establishment.

This grim situation led to the organisation by Galliard, Gurr, Mercer and Rhodes of an international symposium on the 'Chemistry and Biochemistry of Plant Lipids', which was held at the Food Research Institute in Norwich, United Kingdom in 1974. Unwittingly, the organisers of this meeting set in train the establishment of a whole series of biennial plant lipid symposia that continue to this day (see Table 1.1 for a full list of these meetings).

The Norwich meeting was deemed so successful by the participants that four German colleagues, Lichtenthaler, Heinz, Mangold and Tevini, organised a second symposium in Karlsruhe in 1976. The subsequent progress of plant lipid biochemistry from the 1970s to the present day is best documented in the series of proceedings that have been published from each of the biennial International Congresses that have been held up to the present day. These volumes are listed in Table 1.1, together with books published from several additional notable meetings where plant lipids were a major theme. In the 1990s, a National Plant Lipid Cooperative (NPLC) was established in the United States of America to further research, collaboration and information sharing within the community. Despite its name, NPLC soon became an international resource and a link to the expanding series of databases relating to lipid studies. The principal initiative of NPLC was to establish a new series of plant lipid meetings that were held in the United States of America in the intervening years between the biennial International Congresses. No formal proceedings of these meetings, held near Lake Tahoe, California, have been published although abstracts may be available via the NPLC web site (http://www.msu.edu/user/ohlrogge). Rather than attempting to summarise the immense amount of work in plant lipid biochemistry over the past three decades, I will select just a few achievements and try to link these with the subject of the following section, i.e. the molecular genetics revolution of the 1990s. Readers who are interested in further details of progress in lipid biochemistry and physiology during this period are recommended to consult the proceedings of the various meetings and other reference works as listed in Tables 1.1 and 1.2.

Two notable milestones that occurred in Stumpf's lab shortly before his retirement in the early 1980s, were the purification of the separated components of the fatty acid synthase complex (Stumpf and Shimakata, 1983) and the first purification of a plant acyl desaturase (McKeon and Stumpf, 1982). The availability of these and

Table 1.1 Thirty years of Plant Lipid Congresses, 1974–2004

List of International Plant Lipid Congresses and principal organisers

1. 1974, Norwich, UK, Terry Galliard
2. 1976, Karlsruhe, Germany, Hartmut Lichtenthaler
3. 1978, Sweden, Stockholm, Connie Liljenberg
4. 1980, Paris, France, Paul Mazliak
5. 1982, Groningen, The Netherlands, Jan Wintermans
6. 1984, Neuchatel, Switzerland, Paul-Andre Siegenthaler
7. 1986, Davis, California, USA, Paul Stumpf
8. 1988, Budapest, Hungary, Peter Biacs
9. 1990, Wye, UK, Peter Quinn
10. 1992, Djerba, Tunisia, Abelkader Cherif
11. 1994, Paris, France, Jean-Claude Kader
12. 1996, Toronto, Canada, John Williams
13. 1998, Seville, Spain, Juan Sanchez
14. 2000, Cardiff, UK, John Harwood
15. 2002, Okazaki, Japan, Norio Murata
16. 2004, Budapest, Hungary, Peter Biacs

*Published proceedings from the International Plant Lipid Congresses**

Galliard, T. and Mercer, E.J., eds (1975) *Recent Advances in the Chemistry and Biochemistry of Plant Lipids*, Academic Press, London

Tevini, M. and Lichtenthaler, H.K., eds (1977) *Lipids and Lipid-Polymers in Higher Plants*, Springer Verlag, Berlin

Appelqvist, L.A. and Liljenberg, C., eds (1979) *Biochemistry and Physiology of Plant Lipids*, Elsevier, Amsterdam

Mazliak, P., Benveniste, P., Costes, C. and Douce, R., eds (1981) *Biogenesis and Function of Plant Lipids*, Elsevier, Amsterdam

Wintermans, J.F.G.M. and Kuiper, P.J.C. (1982) *Biochemistry and Metabolism of Plant Lipids*, Elsevier, Amsterdam

Siegenthaler, P.A. and Eichenberger, W., eds (1984) *Structure, Function and Metabolism of Plant Lipids*, Elsevier, Amsterdam

Stumpf, P.K., Mudd, J.B. and Nes, W.D., eds (1987) *Metabolism, Structure and Function of Plant Lipids*, Plenum, New York

Biacs, P.A., Gruiz, K. and Kremmer, T., eds (1989) *Biological Role of Plant Lipids*, Plenum, New York

Quinn, P.J. and Harwood, J.L., eds (1991) *Plant Lipid Biochemistry, Structure and Utilization*, Portland Press, London

Cherif, A., Daoud, D., Marzouk, B., Smaoui, A. and Zarrouk. M., eds (1992) *The Metabolism, Structure and Utilization of Plant Lipids*, Centre National Pédagogique, Tunis

Kader, J.C. and Mazlaik, P., eds (1995) *Plant Lipid Metabolism*, Kluwer, Dordrecht

Williams, J.P., Khan, M.U. and Lem, N.W., eds (1997) *Physiology, Biochemistry and Molecular Biology of Plant Lipids*, Kluwer, Dordrecht

Table 1.1 (continued)

Sanchez, J., Cerda-Olmedo, E. and Martinez-Force, E., eds (1998) *Advances in the Biochemistry and Physiology of Plant Lipids*, Universidad de Sevilla, Secretariado de Publicationes

Harwood, J.L. and Quinn, P.J. (2001) *Recent Advances in the Biochemistry of Plant Lipids*, Portland Press, London

Murata, N., Yamada, M., Nishida, I. *et al.* eds (2003) *Advanced Research on Plant Lipids*, Kluwer, Dordrecht

* Dates refer to publication, which is sometimes in the year after the meeting in question.

Table 1.2 Books on Plant Lipids

Other conference proceedings

Thompson, W.W., Mudd, J.B. and Gibbs, M., eds (1983) *Biosynthesis and Function of Plant Lipids*, American Society of Plant Physiologist, Baltimore, USA (6th Ann. Symp. Bot. Riverside, California, 1983)

Murata, N. and Somerville, C., eds (1993) *Biochemistry and Molecular Biology and Storage of Plant Lipids*, American Society of Plant Physiologist, Rockville, USA (US/Japan Binational Seminar, Kona, Hawaii, 1992)

Harwood, J.L. ed (1998) *Plant Lipid Biosynthesis, Fundamentals and Agricultural Applications*, Cambridge University Press, Cambridge (Soc. Exp. Biol. conference, Canterbury, UK, 1997)

Other books related to plant lipids

Gunstone, F.D., Harwood, J.L. and Padley, F.B., eds (1994) *The Lipid Handbook*, 2nd edn, Chapman and Hall, London (This is one of the premiere reference works dealing with lipid composition and analysis.)

Gurr, M.I. and James, A.T. (1971) *Lipid Biochemistry: An Introduction*, Chapman and Hall, London

(Early editions contain many descriptions of experimental techniques and examples applied to plant lipids. The latest is the 5th edition and is written by Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002) Blackwell Science, Oxford.)

Harwood, J.L. and Russell, N.J. (1984) *Lipids in Plants and Microbes*, George Allen and Unwin, London

Hitchcock, C. and Nichols, B.W. (1971) Plant Lipid Biochemistry: The Biochemistry of Fatty Acids and Acyl Lipids with Particular Reference to Higher Plants and Algae

Stumpf, P.K. ed (1980) *Lipids: Structure and Function*, Vol 4, The biochemistry of plants, a comprehensive treatise, Stumpf PK and Conn eds in chief, Academic Press, New York

Moore, T.S. (1993) *Lipid Metabolism in Plants*, CRC Press, Boca Raton

other purified proteins allowed for the initial steps in obtaining amino acid sequence information and/or the preparation of antibody probes, which would eventually lead to the cloning of the corresponding genes. The work on fatty acid synthase was taken further in the 1990s by Slabas *et al.* in Durham, United Kingdom, who refined the purification of the fatty acid synthase components and then collaborated with Rice *et al.* in Sheffield, United Kingdom to use X-ray crystallography to determine