ADVANCES IN PLANT
BIOCHEMISTRY & BIOTECHNOLOGY

ADVANCES IN PLANT CELL BIOCHEMISTRY AND BIOTECHNOLOGY

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PREFACE

Man has been concerned with understanding the processes which occur in the living plant cell since it was realised that the reactions of organic molecules were responsible for life itself. Biochemistry has now developed well beyond that relatively simple concept. Biotechnology, or the application of the understanding of these processes for a practical purpose, is, however, considered by many to be a relatively young branch of science. Yet, the aims of modern biotechnology have been around for centuries. The principles of plant selection for higher yield or taste were precisely the same as biotechnology now tries to achieve through our increased knowledge of the exact biochemical pathways. It is, therefore, sensible to take a more integrated approach to the biochemical and biotechnological aspects of the plant cell.

It would be arrogant and foolish to suggest that this Research Annual series is unique. There are many other review volumes produced on a regular basis which are biochemically and biotechnologically orientated. However, many of these are solely devoted to one or other subject while others include and are dominated by animal or microbiological topics. This series will be devoted purely to plant matters but may spread beyond the bound of its title to include other related disciplines when appropriate.

Plants sythesize a whole host of products which are useful to man in many ways. Primarily they must be considered as sources of nutrients in the form of food. I am sure that attempts have been made by agencies such as FAO to assess the value of plants as sources of food on a world-wide basis. The actual relevance of such statistics is dubious since the need of man and animal life in general is paramount. Even though the Western world has built up food mountains, which include plant materials, the populations of some parts of our planet are still starving. Not only are plants able to provide us with protein and energy, in the form of starch, sugar and lipids, they also provide us, in a balanced diet, with other dietary requirements such as vitamins and essential micro-nutrients.

The presence and requirements of plants for food is not just as simple as that. Some plants contain toxic constituents and these plants have to be avoided or the toxins rendered harmless by some form of treatment. Other plants may be indigestible to man but a simple treatment such as boiling may render them highly nutritious. Furthermore, there are many parts of the world where fresh plant material is not available all the year round. To sustain us during these periods, plants must be grown during the rest of the year and be able to be stored or preserved with the minimum of degradation and damage. On a more esoteric note, taste and texture play important roles in our enjoyment of different foods. Some of these qualities are determined by minor constituents of the plant which may only be secondary metabolites.

Man does not use plants only as a source of food. Although the introduction of man-made fibres in the last 50 years has altered our attitude to textiles, man has made extensive use of natural plant and animal fibres for centuries. Even with all the research effort of multinational companies, it is not yet possible to make fibres with the special qualities of cotton and linen. It should not be forgotten that the man-made fibre industries rely on oil for their raw material. Who can foresee what may happen when many industries compete for diminishing oil supplies. We also, of course, make extensive use of plant fibres as a source of paper and related products. Finally, we must not forget the contribution of timber as a plant product. Perhaps the salient point in all these thoughts is that plants and plant products, whatever their use, are RENEWABLE sources of raw materials.

The first volume of this series contains some very diverse reviews. The authors have been requested to provide as much up-to-date material as possible but not necessarily to make the articles fully comprehensive with considerable historical material. The first volume contains reviews on plant lipids, seed globulins from legumes, the use of plant cells in bioreactors, the importance of tannins and the use of pectins in the food industry. It is hoped that these reviews will challenge the reader with original thought for further advances.

Scottish Crop Research Institute
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Ian M. Morrison
Series Editor

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BIOCHEMISTRY OF PLANT LIPIDS

J.L. Harwood and G. Griffiths

OUTLINE

Advances in Plant Cell Biochemistry and Biotechnology
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1. PLANT LIPIDS: THEIR NATURE AND DISTRIBUTION

Lipids are a very diverse collection of molecules partly because their definition is based on solubility properties rather than any specialized chemical structure. Nevertheless certain molecules are especially prominent in Nature and this includes the plant kingdom. In this review we will concentrate on acyl lipids (i.e. those containing fatty acid moieties) since these are quantitatively by far the most important. Nevertheless one should remember that compounds such as the chlorophyll or carotenoid pigments, sterols and a wide range of terpenoids have important functions and may, in certain tissues, be major components.

Obviously, the building blocks of acyl lipids are fatty acids. Because of their route of biosynthesis, molecules with even numbers of carbon atoms predominate. In higher plants, over 90% of the total fatty acids of leaves are represented by seven fatty acids while these same acids constitute about 90% for the total of edible oils. The fatty acids concerned are the saturated molecules laurate, myristate, palmitate and stearate, and the C18 unsaturated molecules oleate, linoleate and linolenate [1, 2]. In algae, particularly marine species, the C20 acids, arachidonate and eicosapentaenoate are often major constituents [3]. Common plant acids are listed in Table 1 together with some notes about their occurrence and function.

The structure of the above named unsaturated fatty acids emphasizes the point that double bonds are usually separated from each other by a methylene grouping. In addition, the double bonds are almost invariably in the *cis* (or (*Z*) form) configuration. Nevertheless, acids with conjugated or *trans* (or (*E*) form) double bonds do occur [2]—especially in storage oils, as intermediates in metabolism, or as products of oxidation.

One point which has been made again and again is the strikingly similar fatty acid composition of leaf tissues (and probably also of shoots or roots) from different plants. This consistency of composition contrasts markedly with the variability of seed contents. Seeds can contain 90% or so of their fatty acids as a single unusual component, e.g. ricinoleate in castor bean. This point is shown clearly in Table 2 where the fatty acid content of a range of plant leaves is compared with that of a range of commercial seeds.

Table 1. The common fatty acids of plants.

Fatty acid	Systematic name	Comments
Lauric	dodecanoic	widespread minor component but major in palm and coconut fruits
Myristic	tetradecanoic	widespread minor component
Palmitic	hexadecanoic	the main saturated fatty acid of plants
Stearic	octadecanoic	widespread minor component, occasionally major as in cocoa butter
Oleic	*cis*-9-octadecenoic	the main monounsaturated fatty acid of algal and plant tissues; also a major component of commercially important edible oils
Linoleic	*cis,cis* -9,12-octadecadienoic	a major component of all plant tissues; by far the most common polyunsaturated acid of edible oils
α-Linolenic	all *cis*-9,12,15-octadecatrienoic	the main fatty acid of leaves; usually minor component of seed oils but occasionally major, e.g. linseed
Arachidonic	all *cis*-5,8,11,14-eicosatetraenoic	not usually found in significant amounts in higher plants but major component of mosses, liverworts and, especially, marine algae

There is no evidence for the widespread occurrence of either plasmalogens or sphingolipids in plants and most fatty acids are, therefore, linked to glycerolipids through ester bonds. Two classes of glycerolipids are important. These are the phosphoglycerides and the glycosylglycerides. The same phosphoglycerides that are found in other eukaryotes occur in plants but their relative distribution may be rather different (this point is covered below in the discussion of membrane composition). In photosynthetic tissues phosphatidylglycerol is the main phospholipid with phosphatidylcholine, phosphatidyl-

Table 2. Comparison of the fatty acid composition of plant leaves with that of different seed oils

Tissue	Fatty acid content (% total)						
	<C16	16:0	18:0	18:1	18:2	18:3	others
Leaves	trace-2	10-13	1-4	3-7	8-15	53-67	2-4
Seed oils							
palm kernal	79	6	2	10	2	trace	1
coconut	84	7	2	5	1	trace	1
palm	1	45	5	39	10	trace	trace
cocoa butter	trace	26	34	35	3	trace	2
olive	trace	10	2	78	7	1	2
safflower	trace	6	3	14	75	1	1
linseed	trace	6	3	17	14	60	trace
castor bean	trace	1	1	3	4	trace	91

ethanolamine and phosphatidylinositol also important. The amount of cardiolipin (or diphosphatidylglycerol), which is a component of mitochondrial inner membranes, reflects the tissue content of this organelle. Until recently it was thought that the higher inositides did not occur in plants. However, several groups of workers [4-6] have now identified phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bis-phosphate in a range of tissues. It seems likely, therefore, that these are widespread but minor phospholipid constituents in plants.

To those readers only familiar with animal or microbial lipids, the presence of large amounts of glycosylglycerides in plant tissues will be something of a novelty. In fact, because of the large amounts of monogalactosyldiacyglycerol in chloroplast thylakoid membranes, this compound is actually the most abundant lipid in Nature [7] in spite of being rarely mentioned in student biochemistry text books! Three glycosylglycerides are important in plants. These are monogalactosyldiacylglycerol, digalactosyldiacylglycerol and sulphoquinovosyldiacylglycerol (also known less precisely as the plant sulpholipid). The structures of these three glycosylglycerides are shown in Figure 1.

Whereas phospholipids and glycosylglycerides function mainly as membrane constituents, the usual storage lipid is triacylglycerol (though the commercially important jojoba oil is actually a wax ester).

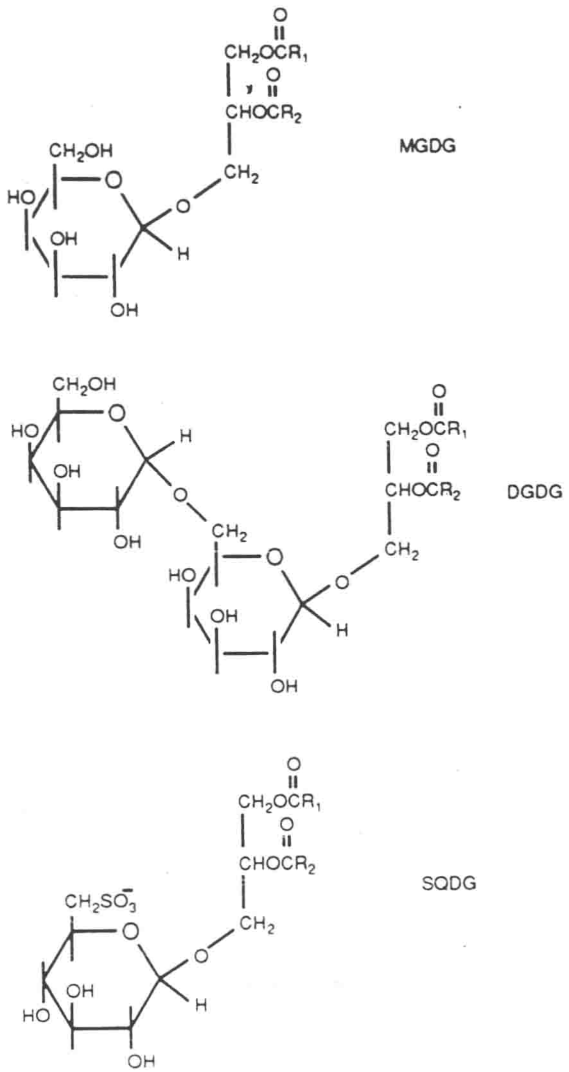

Figure 1. Structures of the chloroplast glycosylglycerides MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulphoquinovosyldiacylglycerol.

In addition, the surface coverings of plants contain much lipid but of a different and rather ill-defined composition. The aerial portions of plants are covered in cutin while the subterranean parts (as well as wounds) are covered in suberin. Although both cutin and suberin contain many very long chain components, they differ in the relative pro-

Table 3. The important features of the composition of cutin and suberin. See Kolattukudy [8] for more details.

Component	Cutin	Suberin
Very long-chain fatty acids	rare and minor	common and substantial
Very long-chain alcohols	rare and minor	common and substantial
In-chain substituted acids	major	minor (sometimes substantial)
Dicarboxylic acids	minor	major
Phenolics	low	high

portions of the various constituents. A simplified description of their composition is given in Table 3.

In addition to the compounds listed in Table 3, a number of other lipids are important in plant surface coverings. These include hydrocarbons, ketones, secondary alcohols, aldehydes, diketones and di- or polyesters.

Analyses based on the extraction of whole tissues may, to some extent, be misleading because they fail to take account of the detailed differences which may exist between different cells or between different parts of a given cell. Accordingly, it is preferable to be able to analyse the lipid composition of, for example, a particular membrane. There are many reports of such analyses in the literature. However, there are some difficulties in comparing data. For example, the lipid composition of a given membrane (say plasma membrane) from different plants may not be particularly similar so that comparisons of, say, plasma membranes from potato tubers with glyoxysomal membranes from castor bean are not usually valid. For comparative purposes, only membranes from the same type of tissue should be considered. As a further complication, the composition of a particular membrane changes with development and is subject also to environmental influence. Finally, a number of publications do not show how pure the membrane preparations are so that the analytical results quoted could reflect a high degree of contamination. As an example, reports of diphosphatidylglycerol in fractions other than the inner mitochondrial membrane are almost certainly due to contamination [8]. Nevertheless, if one bears the above considerations in mind, one can still make some general conclusions about the differences in lipid composition between membranes.

Some examples of lipid composition of non-chloroplast membranes are shown in Table 4. Sterols and their (acyl) glycosides are rather minor components of plant membranes. They tend to be most prevalent in the plasma membrane. Phosphatidylcholine is the major lipid with phosphatidylethanolamine also important in all membranes. Phosphatidylglycerol and phosphatidylinositol are significant components in each case while the phosphorylated inositides are found in plasma membranes. Diphosphatidylglycerol (cardiolipin) is exclusively located on the inner mitochondrial membrane [9] where it can actually be used, instead of enzyme activity, as a marker for the assessment of the efficiency of subcellular fractionations.

Table 4. Some examples of the phospholipid compositions of different non-plastid plant membranes

	Phospholipid composition (wt%)				
Membrane	PC	PE	PG	PI	DPG
Plasma membrane[b]	32	46	0	19	3
Mitochondrial(outer)[a]	51	39	trace	10	0
Mitochondrial(inner)[a]	34	58	1	2	7
Glyoxysomal[a]	48	36	8	4	0
Glyoxysomal[b]	61	20	15	4	0
Microsomes[b,d]	45	33	2	16	1
Microsomes[c,b,d]	67	14	trace	19	0

[a] Castor bean. [b] Potato. [c] Pea. [d] Mainly endoplasmic reticulum.

In comparison to the phospholipid dominated membranes listed in Table 4, the thylakoid membranes of higher plants, algae and cyanobacteria are dominated by the presence of glycosylglycerides and, of course, pigments [10]. Phosphatidylglycerol is the main, in some cases the only, phospholipid. The envelope membranes which surround the chloroplast are frequently analysed as a total fraction, when they appear to contain roughly equal amounts of phosphoglycerides and glycosylglycerides. However, it is now apparent that the inner envelope lipid composition broadly resembles the thylakoids while the outer envelope is similar to the endoplasmic reticulum [11] with which it has been claimed to be continuous [12].

The lipids of non-plastid membranes usually have palmitate, oleate,