Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

## THE LIPID METABOLISM OF PLANTS

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry at Massey University.

by

Philip Grattan Roughan

1968

#### ABSTRACT

A method, based on the isolation of pure compounds by a combination of DEAE-cellulose and thin-layer chromatography, has been developed for the rapid and quantitative estimation of the major glycerolipids of plant tissues. The method has been used 1) for the analyses of the major glycerolipids of a wide variety of plant species and 2) as part of a detailed chromatographic analysis of the glycerolipid constituents of the green alga Mesotaenium caldariorum during which two unknown lipids, probably glycerolipids, were isolated.

On the basis of the incorporation of radiocarbon from <sup>14</sup>C-labelled precursors into the glycerolipids of both Mesotaenium cells and pumpkin leaves, the likely-hood of relatively low turnover rates for the various glycerolipids, with the possible exceptions of phosphatidyl glycerol in Mesotaenium and phosphatidyl choline in pumpkin leaf, is discussed.

The unusual growth requirements of <u>Mesotaenium</u> caldariorum in liquid culture is discussed briefly.

#### PREFACE

In this study, a considerable amount of time has been spent on the development of techniques for the routine separation and analysis of all of the major glycerolipids of plant tissues. These techniques were considered an essential prerequisite for obtaining the type of results envisaged in the planning of the topic. Time limitations have subsequently dictated that a lesser period than would have been desired was available for metabolic studies, so that the isotope incorporation experiments reported here should be regarded as preliminary in nature. Nonetheless, these experiments do point the way for further investigations which could provide reliable measurements of the turnover of the individual glycerolipids of algae and leaves.

I wish to express my appreciation to Professor R.D. Batt for his advice and guidance during the course of this work and to Dr K.J. Mitchell for his constant encouragements. To Dr A.O. Taylor go my thanks for his patience and attentiveness during our discussions of some aspects of this work.

I am indebted to the Department of Scientific and Industrial Research for ensuring me employment at the Plant Physiology Division of the Department while this investigation was carried out.

P.G. Roughan.

## TABLE OF CONTENTS

## PART 1

# THE IDENTIFICATION, SEPARATION AND ANALYSIS OF PLANT GLYCEROLIPIDS

#### INTRODUCTION

•	The glycerolipids of photosynthetic tissue.	1	
•	Extraction of lipids.	2	
•	Removal of non-lipid.	3	
•	Qualitative analyses.	5	
•	Quantitative analyses.		
•	Summary.	11	
	Aim of the present investigation.	12	
	METHODS	14	
•	Selection of plant material.	14	
•	Extraction and purification of lipid.	15	
•	Preliminary separation of glycerolipids.	16	
•	Thin-layer chromatography.	19	
•	Quantitative estimation of phospholipids.	25	
•	Analysis of glycolipids.	28	
•	Application of the new method to a variety		
	of species.	33	
•	Chemicals.	34	

# RESUL'IS

1.	Lipid extraction and purification.	35			
2.	Preliminary separations.				
3.	Thin-layer chromatography.				
4.	Quantitative analyses of phospholipids.	40			
5.	Quantitative analyses of glycolipids.	41			
6.	Simultaneous analyses of glyco- and				
	phospholipids.	46			
7.	Glycerolipid composition of a variety of				
	species.	47			
	DIGGLOSTON				
	DISCUSSION	F.C			
1.	The new technique.	56			
2.	The glycerolipids of different species.	63			
	PART 2				
	THE GLYCEROLIPIDS OF MESOTAENIUM				
	CALDARIORUM AND THEIR TURNOVER				
		<b>6</b> -			
	INTRODUCTION	67			
	METHODS				
1.	Growth conditions.	74			
2.	Analysis of growth.	75			
3.	Measurement of photosynthetic potential.				
+•	Glycerolipid analyses.	78			
5.	Fatty acid analyses.	80			

6.	14C-Incorporation studies.				
7.	Measurement of radioactivity.				
	RESULTS				
1.	Growth of Mesotaenium caldariorum in				
	liquid culture.	83			
2.	The lipids of M. caldariorum.				
3.	3. Isotope incorporation studies.				
	DISCUSSION				
1.	Growth of M. caldariorum.	122			
2.	Glycerolipids of Mesotaenium.	123			
3.	Fatty acids.	126			
4.	Glycerolipid, 14C-incorporation studies.	127			
	PART 3				
	TURNOVER OF THE GLYCEROLIPIDS OF CUCURBITER LEAVES				
	INTRODUCTION	133			
	METHODS	135			
	RESULTS	139			
1.	. Incorporation studies using squash leaves				
	a) Using detached leaves.	140			
	b) Using intact leaves.	141			

•	Turnove	r studies using pumpkin leaves.	
	a) Usin	g 14co <sub>2</sub> .	
	b) Using	g <sup>14</sup> C-acetate.	147
•	Labelli	ng of fatty acids in PC and MGD	
	of pumpl	kin leaves.	154
	DISCUSS	ION	159
	REFERENC	CES	162
PPEI	DIX 1	An examination of a recently	
		published method for the quantitative	
		estimation of plant sulpho- and	
		galacto- lipids.	i
PPEI	MDIX 2	Some notes on the lipid extraction	
		and hydrolysis methods used in a	
		published method for the analysis	
		of lipid-bound galactose.	xxii

)LD OUT Abbreviations used.

PUBLICATIONS arising from material in this thesis.

- Simple devices for the application of samples as narrow streaks for thin-layer chromatography.
   P.G. Roughan and C.G. Tunnicliffe (1967).
   J. Lipid Res. 8: 511.
- Quantitative analysis of sulfolipid (sulfoquinovosyl diglyceride) and galactolipids (monogalactosyl and digalactosyl diglycerides) in plant tissues.
  P.G. Roughan and R.D. Batt (1968).
  Anal. Biochem. 22; 74.
- The glycerolipid composition of leaves.
  P.G. Roughan and R.D. Batt (1969).
  Phytochemistry, 8; In Press.