Phytochemistry, 1969, Vol. 8, pp. 1489 to 1494. Pergamon Press. Printed in England.

THE LEAF LIPIDS OF SOME MEMBERS OF THE BORAGINACEAE FAMILY

G. R. JAMIESON and E. H. REID

Chemistry Department, The Paisley College of Technology, Paisley, Scotland

(*Received* 3 *January* 1969)

Abstract—Leaf lipids of five members of the Boraginaceae contain relatively high proportions of γ-linolenic and octadecatetraenoic acids. Octadecatetraenoic acid is concentrated mainly in the monogalactosyl diglyceride fraction of *Myosotis scorpioides* leaf lipids. Variations in the proportions of polyunsaturated acids are found during the growing season. A large number of branched-chain saturated acids are found as trace components of *M. scorpioides* leaf lipids.

INTRODUCTION

AS A RESULT of investigations into the composition of leaf lipids it has been established[1] that there is a close similarity in lipid composition of all deciduous leaf tissues. During a preliminary survey of various marsh and water plants Jamieson and Reid[2] found that the leaf lipids of *Myosotis scorpioides* contained relatively large proportions of γ-linolenic (18:3ω6) and octadecatetraenoic (18:4ω3) acids. Both these acids have been found in the seed oils of some members of the Boraginaceae.[3–5]

It was of interest to investigate the changes in fatty acid composition of leaf lipids during the growing season with special reference to the polyunsaturated acids which are the major constituent acids of chloroplast lipids, since Klopfenstein and Shigley[6] have indicated that, as a plant matures, its leaf lipids become more saturated.

Although there have been recent investigations into the minor component saturated acids of marine lipids[7–11] there has been little interest in similar components of plant lipids. Ackman and Hooper[11] have shown that, with the use of silicic acid–silver nitrate column chromatography and gas–liquid chromatography on high efficiency open-tubular columns, the minor saturated acids of fish oils may be examined in detail. Similar methods have been used in the present work to investigate the minor saturated acids of *M. scorpioides* leaf lipids.

RESULTS AND DISCUSSION

The fatty acid composition of the total lipids from leaves of five species of Boraginaceae is shown in Table 1. There is a close similarity in fatty acid composition among the five

[1] B. W. NICHOLS, *Phytochem.* **4**, 769 (1964).
[2] G. R. JAMIESON and E. H. REID, *J. Sci. Food Agric.* **19**, 628 (1968).
[3] B. M. CRAIG and M. K. BHATTY, *J. Am. Oil Chem. Soc.* **41**, 209 (1964).
[4] C. R. SMITH, JR., J. W. HAGEMANN and I. A. WOLFF, *J. Am. Oil Chem. Soc.* **41**, 290 (1964).
[5] R. KLEIMAN, F. R. EARLE, I. A. WOLFF and Q. JONES, *J. Am. Oil Chem. Soc.* **41**, 459 (1964).
[6] W. E. KLOPFENSTEIN and J. W. SHIGLEY, *J. Lipid Res.* **8**, 350 (1967).
[7] R. G. ACKMAN and J. C. SIPOS, *Comp. Biochem. Physiol.* **15**, 445 (1965).
[8] R. G. ACKMAN, J. C. SIPOS and C. S. TOCHER, *J. Fish. Res. Bd. Canada* **24**, 635 (1967).
[9] R. G. ACKMAN and R. P. HANSEN, *Lipids* **2**, 357 (1967).
[10] R. G. ACKMAN, C. A. EATON and S. N. HOOPER, *Can. J. Biochem.* **46**, 197 (1968).
[11] R. G. ACKMAN and S. N. HOOPER, *Comp. Biochem. Physiol.* **24**, 549 (1968).

species, the major components being palmitic, linoleic, γ-linolenic, linolenic, and octadecatetraenoic acids. The sum of these acids accounts for 74–91 per cent of the total acids present. In general it has been found[12] that the major fatty acids of leaf lipids are linolenic, linoleic, and palmitic acids and γ-linolenic and octadecatetraenoic acids have so far only been found in the lipids from the photosynthetic tissue of mosses and ferns.[1,13,14] The presence of γ-linolenic and octadecatetraenoic acids in the leaf lipids of the Boraginaceae species studied could be of great taxonomic interest, since the only other example of leaf lipids containing significant quantities of unusual fatty acids occurs in Malvaceae and Sterculiaceae which contain cyclopropenoid fatty acids.[15]

TABLE 1. FATTY ACID COMPOSITION OF LEAF LIPIDS OF FIVE SPECIES OF THE BORAGINACEAE

Acid	*Myosotis scorpioides*	*Myosotis arvensis*	*Myosotis alpestris*	*Symphytum officinale*	*Pulmonaria officinalis*
12:0	0·1	0.2	0·2	tr.	0·2
13:0	0·2	0·1	0·1	0·1	0·1
14:0	0·7	0·9	0·8	0·3	0·4
15:0	0·1	0·1	0·2	0·2	0·1
16:0	14·8	14·1	13·2	10·3	11·4
16:1*	1·7	1·0	1·0	2·3	0·9
17:0	0·1	0·1	0·1	0·1	0·1
18:0	1·7	2·3	1·5	1·4	1·1
18:1†	2·1	10·0	5·3	2·2	4·8
18:2ω6	18·8	22·5	14·3	12·7	16·9
18:3ω6	14·0	12·1	5·1	3·2	6·0
18:3ω3	23·9	17·6	34·0	52·1	38·5
18:4ω3	14·5	7·3	20·2	12·9	17·5
20:0	1·2	2·0	0·8	0·4	0·4
20:1	0·3	1·8	0·6	0·3	0·6
21:0	0·1	0·1	0·1	0·1	0·2
22:0	2·5	3·3	1·6	0·6	0·4
22:1	0·2	0·4	tr.	0·2	0·1
23:0	0·1	0·2	tr.	0·2	0·1
24:0	2·3	3·1	0·9	0·3	0·2
24:1	0·6	0·8	tr.	0·1	tr.

* Both ω9 and ω7 isomers present.
† Both ω6 and ω9 isomers present.
tr. = trace.

Jamieson and Reid[2] have shown that there is a variation in fatty acid composition of *Myosotis scorpioides* leaf lipids during the middle and last part of the growing season and these results have been extended to include samples taken in the spring and early summer from both sheltered and exposed localities. The samples for each analysis were aggregates from a number of plants and consisted of leaves at different stages of development. The variations in proportions of the major C_{18} acids during April to December are shown in Fig. 1. Although the variations are similar for both the sheltered and exposed locality samples the changes are more extreme in the sheltered locality samples. Both linolenic and octadecatetraenoic

[12] B. W. NICHOLS and A. T. JAMES, in *Progress in Phytochemistry* (edited by L. REINHOLD and Y. LIWSCHITZ), Vol. 1, p. 1, Interscience, London (1968).
[13] J. L. GELLERMAN and H. SCHLENK, *Experientia* **20**, 426 (1964).
[14] H. SCHLENK and J. L. GELLERMAN, *J. Am. Oil Chem. Soc.* **42**, 504 (1965).
[15] F. S. SHENSTONE and J. R. VICKERY, *Nature* **190**, 168 (1961).

acids reach minimum proportions during August–September, whilst linoleic acid reaches maximum proportions in September. There is not such a variation in the γ-linolenic acid

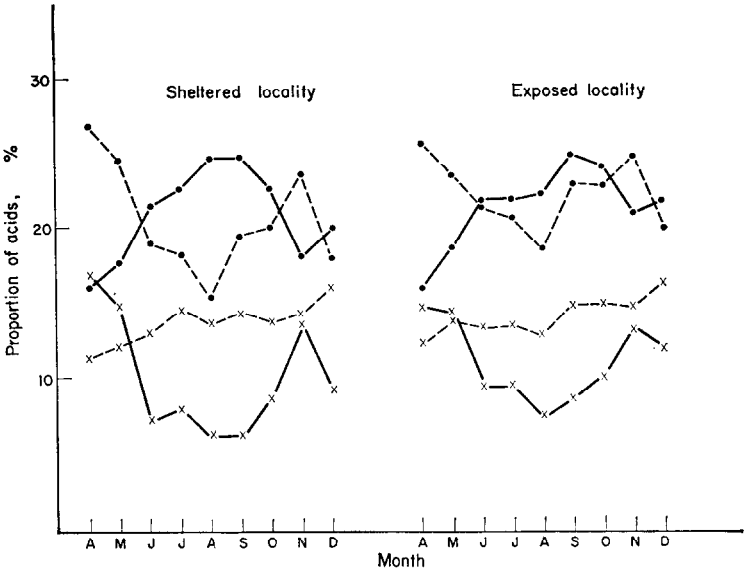

FIG. 1. VARIATIONS IN PROPORTIONS OF C_{18} POLYUNSATURATED ACIDS DURING THE GROWING SEASON OF *Myosotis scorpioides*.

×——× 18:4ω3 ×----× 18:3ω6
●——● 18:2ω6 ●---● 18:3ω3

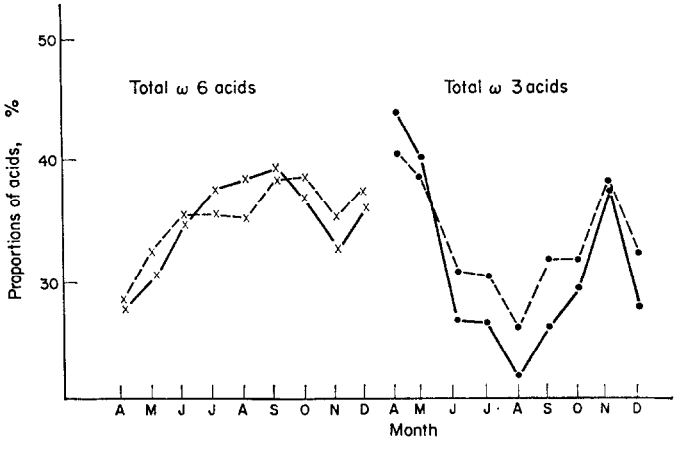

FIG. 2. VARIATIONS IN PROPORTIONS OF TOTAL ω6 AND TOTAL ω3 ACIDS DURING THE GROWING SEASON OF *Myosotis scorpioides*.

——— sheltered locality --- exposed locality.

content which gradually increased from April to December. It is intended to continue this study by analysing leaves at different stages of development.

The variations in proportions of ω6 and ω3 acids are shown in Fig. 2. The total ω6 acids reach maximum proportions in September–October and the total ω3 acids minimum

proportions in August. The ratio of total ω6 acids: total ω3 acids reaches a maximum in August, the end of the flowering season in this district. Klopfenstein and Shigley have reported[6] that the sulpholipids and phospholipids of lucerne become more saturated during maturation. The proportion of saturated acids of *M. scorpioides* total lipids reaches a maximum during the flowering season of the plant.

It has been found[16–18] that, in various plant tissues, there is a preferential concentration of the more highly unsaturated fatty acids in the galactosyl diglycerides. In green tissues of higher plants and in photosynthetic micro-organisms linolenic acid is the major constituent of the chloroplast lipids. Since the Boraginaceae species studied contain relatively high proportions of the polyunsaturated, linolenic, γ-linolenic, and octadecatetraenoic acids, it is

TABLE 2. FATTY ACID COMPOSITION OF DIFFERENT LIPID CLASSES OF *Myosotis scorpioides*

Acid	T	N	MGDG	DGDG	P
12:0	tr.	0·5	tr.	tr.	0·3
13:0	0·3	1·0	—	—	—
14:0	1·0	3·6	0·2	0·3	0·4
15:0	0·2	0·6	—	tr.	tr.
16:0	13·6	17·4	3·0	18·6	40·9
16:1	1·2	tr.	0·2	1·7	7·5
17:0	0·1	0·2	—	0·1	0·1
18:0	1·3	2·6	0·4	2·5	3·3
18:1	2·2	4·7	0·7	1·8	3·9
18:2ω6	16·7	18·5	3·4	10·9	23·1
18:3ω6	12·0	16·9	6·1	7·9	6·8
18:3ω3	28·0	8·8	41·5	43·4	7·8
18:4ω3	18·7	9·4	44·0	11·9	1·5
20:0	0·2	0·6	—	tr.	0·4
20:1	0·6	0·2	—	0·2	0·8
21:0	0·1	0·2	—	—	0·1
22:0	1·9	7·8	0·3	0·2	1·6
22:1	tr.	0·4	—	—	0·1
23:0	tr.	0·1	—	—	0·2
24:0	1·4	6·1	tr.	tr.	0·5
24:1	0·5	0·4	tr.	tr.	0·7

T: total lipids; N: neutral lipids; MGDG: monogalactosyl diglycerides; DGDG: digalactosyl diglycerides; P: more polar lipids; tr.: trace.

of interest to examine the distribution of these acids among the different lipid classes. The fatty acid composition of four classes of *M. scorpioides* lipids is shown in Table 2. Linolenic acid is concentrated in the monogalactosyl and digalactosyl diglycerides and octadecatetraenoic acid in the monogalactosyl diglycerides. Linolenic acid plus octadecatetraenoic acid account for 85·5 and 55·3 per cent of the monogalactosyl diglycerides and digalactosyl diglycerides respectively. γ-Linolenic acid is distributed among all the lipid classes, the highest proportion being in the neutral fraction.

Particular interest has been shown recently in the occurrence and biochemical significance of the three isoprenoid acids, 4,8,12-trimethyltridecanoic (4,8,12-TMTD), pristanic, and phytanic acids and it has been established that terrestial mammals and rumen micro-

[16] J. S. O'BRIEN and A. A. BENSON, *J. Lipid Res.* **5**, 432 (1964).
[17] E. LEVIN, W. J. LENNARZ and K. BLOCH, *Biochim. Biophys. Acta* **84**, 471 (1964).
[18] A. ROSENBERG, J. GOUAUX and P. MILCH, *J. Lipid Res.* **7**, 733 (1966).

organisms convert phytol into phytanic acid. Phytol has been suggested as the common precursor of most of the isoprenoid materials so far identified in marine life. There is little information on the minor component saturated acids of plant lipids.

The saturated methyl esters were obtained from *M. scorpioides* total lipid methyl esters

TABLE 3. EQUIVALENT CHAIN LENGTHS (ECL) AND COMPOSITION OF SATURATED FATTY ACIDS OF *Myosotis scorpioides*

	Composition %					
ECL	Normal	iso	anteiso	X	Y	Z
11·65		0·01				
12·00	0·13					
12·30					0·53	
12·65		0·09				
13·00	0·09					
13·10				0·08		
13·19				0·10		
13·38					0·02	
13·48						0·08
13·62		0·03				
13·81			0·14			
14·00	3·09					
14·19				0·03		
14·32					0·06*	
14·49						0·02
14·63		0·14				
14·79			0·08			
15·00	0·38					
15·20				0·07		
15·29					0·09	
15·50						0·01
15·61		0·05				
15·78			0·03			
16·00	86·01					
16·32					0·26†	
16·59		0·16				
16·77			0·05			
17·00	0·80					
17·35					0·46‡	
17·60		0·08				
17·75			0·07			
18·00	6·76					
Σ	97·26	0·56	0·37	0·28	1·42	0·11

X, FCL 0·10–0.20; Y, FCL 0·29–0·38; Z, FCL 0·48–0·50.
* Methyl trimethyltridecanoate.
† Methyl pristanate.
‡ Methyl phytanate.

by chromatography on a silicic acid–silver nitrate column and they were examined in detail in the range C_{12}–C_{18} on a high-efficiency Apiezon L open tubular column. The relative proportions of these unsaturated acids are shown in Table 3. As well as normal, iso and anteiso compounds of various chain lengths, three other groups of methyl esters having fractional chain lengths (FCL): (X) 0·10–0·20, (Y) 0·29–0·38; (Z) 0·48–0·50, were found to be present.

Methyl phytanate was tentatively identified from the chromatogram by comparison with an authentic sample[19] and methyl pristanate, and methyl 4,8,12-TMTD by comparison with the retention data of Ackman and Hooper.[11] These three isoprenoid acids have FCL values in group (Y). Ackman, Sipos and Tocher[8] reported a series of methyl esters from marine oils having FCL 0·34 on BDS, and Sano[20] reported two series having FCL of 0·2 and 0·4 on PEGA.

EXPERIMENTAL

Leaves were collected from various areas in the surrounding district of Paisley, and the leaf lipids were extracted and concentrated by the methods described previously.[2] Lipids were separated into classes using silicic acid column chromatography using the solvent system described by Constantopolous and Bloch;[21] fractions from the column were monitored by TLC on silica gel with acetone as eluting solvent.[22] Fatty acid methyl esters were prepared from each of the lipid classes by reaction with BF_3–CH_3OH using the procedures described by Morrison and Smith.[23]

GLC analyses of the total lipid methyl esters and the methyl esters from each of the lipid classes were carried out on a PE 800 chromatograph employing both BDS packed and EGSS-X open tubular columns. The methyl esters were tentatively identified from the chromatograms by comparison with known esters and by the use of separation factors.[24, 25]. The saturated methyl ester fraction from a silicic acid–silver nitrate column was separated on an Apiezon L open tubular column at 210°.

Acknowledgements—Thanks are due to the Science Research Council and to the Chemical Society for financial support.

[19] G. R. JAMIESON, in *Topics in Lipid Chemistry* (edited by F. D. GUNSTONE), Vol. 1, Logos Press, London, in press.
[20] Y. SANO, *Yukagaku* **15**, 140 (1966).
[21] G. CONSTANTOPOLOUS and K. BLOCH, *J. Bacteriol.* **93**, 1788 (1967).
[22] H. W. GARDNER, *J. Lipid Res.* **9**, 139 (1968).
[23] W. R. MORRISON and L. M. SMITH, *J. Lipid Res.* **5**, 600 (1964).
[24] R. G. ACKMAN and R. D. BURGHER, *J. Chromatogr.* **11**, 185 (1963).
[25] G. R. JAMIESON and E. H. REID, *J. Chromatogr.* **26**, 8 (1967).