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LIPIDS CONTAINING VERY LONG CHAIN MONOUNSATURATED ACYL MOIETIES IN SEEDS OF *LUNARIA ANNUA*

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Abstract—Lipids in developing as well as mature seeds of *Lunaria annua* are mainly composed of triacylglycerols which contain almost exclusively nervonoyl (24:1), erucoyl (22:1) and oleoyl (18:1) moieties. Maturation of the seeds proceeds with successive reduction in the relative proportions of phospholipids and glycolipids as well as linoleoyl (18:2) and linolenoyl (18:3) moieties in the total lipids. Concomitantly, the most predominant fraction of triacylglycerols, which contain nervonoyl and erucoyl moieties at the *sn*-1,3 positions and oleoyl moieties at the *sn*-2 position, are accumulated.

INTRODUCTION

Very long chain monounsaturated fatty acids, e.g. gadoleic (20:1) and erucic (22:1) acids, are known to be the major constituents of triacylglycerols of many cruciferous seeds [1]. Search for potential sources of very long chain fatty acids has revealed that the seeds of the crucifer, *Lunaria annua* (honesty), contain unusually high levels of nervonic acid (24:1) besides erucic acid in the total lipids [2, 3]. We report here the composition of the lipid classes in developing as well as mature seeds of *L. annua*.

RESULTS AND DISCUSSION

Developing seeds of *L. annua* at two different stages of maturation (sample I, 4–5 weeks after flowering; sample II, 6–7 weeks after flowering) and the mature seed were investigated. Table 1 records the lipid content and fatty acid composition of the three seed samples. The data show that in the constituent fatty acids of the lipids in the mature seed more than 90% are made up of (n − 9)-monounsaturated fatty acids of which over 70% are composed of 24:1 and 22:1. Large proportions of 24:1 and 22:1 are also present as constituent fatty acids of the lipids in both samples of developing seed.

In order to establish the identity of the monounsaturated fatty acids, each of the fractions of methyl 18:1, methyl 20:1, methyl 22:1 and methyl 24:1 were isolated from the methyl esters derived from the total lipids of the seed samples by TLC and prep. GC. Subsequent analysis of these methyl ester fractions by reductive ozonolysis and GC revealed that each monounsaturated fatty acid is composed exclusively of the (n − 9)isomer (data not shown). It should be noted in this context that seeds of several Cruciferae have been found to contain the unusual (n − 7)monounsaturated fatty acids besides the predominating (n − 9)isomers [4, 5].

The data given in Table 1 also show that progressive maturation of the *L. annua* seed is accompanied by a steady decrease in the levels of 16:0, linoleic (18:2) and linolenic (18:3) acids, and a steep rise in the relative proportions of 24:1 and 22:1, whereas the level of oleic acid (18:1) is barely altered. These patterns of compositional changes with progressive seed maturation broadly resemble those observed in developing seeds of rape [6, 7], crambé [6, 8] and white mustard [9, 10]. They corroborate the biosynthetic pathways observed in seeds of several higher plants, where 18:1 synthesized *de novo* has been found to be elongated to very long chain monounsaturated fatty acids [11–16]. The occurrence of minor proportions of 20:1 in the constituent fatty acids of the developing seeds, and its near disappearance in the mature seed (Table 1) indicates that 20:1 is formed as an

Table 1. Lipid content and fatty acid composition of total lipids of *L. annua* seeds

	Sample		
	Developing seed I†	Developing seed II†	Mature seed
Lipid content (% fr wt)	4.7	5.7	27.2
Fatty acid* (wt%)			
16:0	4.9	3.9	1.3
16:1	0.4	0.3	0.2
18:1	26.0	23.3	23.5
18:2	14.6	12.0	5.7
18:3	5.8	4.1	0.6
20:1	5.1	4.7	0.7
22:1	31.3	36.8	42.7
24:1	11.1	14.4	24.7
18:0–24:0	0.8	0.5	0.6

*Fatty acids are designated by the number of carbon atoms: number of double bonds.

†Age of seeds: I, 4–5 weeks after flowering; II, 6–7 weeks after flowering.

intermediate in the chain elongation of 18:1, whereas 24:1 and 22:1 are the major end products of the elongation reaction in *L. annua* seed.

The distribution of major lipid classes and the composition of their constituent fatty acids in the three samples of *L. annua* seed is shown in Table 2. The data show a decrease with progressive seed maturation in the relative proportion of phospholipids plus glycolipids and concomitant accumulation of triacylglycerols, which are by far the most predominant lipid constituents in each seed sample. In addition, minor proportions of monoacylglycerols, diacylglycerols and unesterified fatty acids are detected in each sample. The pattern of changes in fatty acid composition of total lipids with progressive seed maturation (Table 1) is also broadly reflected in the alterations of fatty acid composition of the individual lipid classes, as shown by the data given in Table 2.

The results show furthermore that in both developing and mature seeds 24:1 and 22:1 are the most abundant constituent fatty acids of the triacylglycerols. The levels of 22:1 and 24:1 are of the decreasing order in unesterified fatty acids, diacylglycerols and monoacylglycerols, whereas the fraction consisting of phospholipids and glycolipids contain only minor proportions of these very long chain acyl moieties. For the developing seed II, the fraction consisting of phospholipids and glycolipids has been further resolved by TLC into individual lipid classes and the composition of their acyl moieties determined (Table 3). These data show that each of the individual classes of phospholipids and glycolipids contain only

Table 2. Composition of lipid classes in *L. annua* seeds

Sample	Lipid class*	% of total lipids	Fatty acid† composition (wt %)							
			16:0	18:1	18:2	18:3	20:1	22:1	24:1	Others‡
Developing seed I	PL + GL	11.6	17.6	26.1	29.1	23.1	0.9	0.6	0.9	1.7
	MG	0.6	13.9	31.8	28.0	12.5	1.1	2.1	3.2	7.3
	DG	1.3	9.2	36.7	22.7	8.0	4.3	12.6	4.2	2.3
	FA	0.7	5.9	41.9	11.9	2.7	6.1	20.7	6.8	4.0
	TG	85.8	2.1	27.1	11.9	2.4	5.3	37.7	12.6	0.9
Developing seed II	PL + GL	5.5	15.2	25.1	24.7	26.4	0.8	2.8	2.4	2.6
	MG	0.5	13.7	35.5	28.3	10.3	1.7	2.5	2.0	6.0
	DG	0.6	8.5	34.4	15.5	4.6	3.6	21.0	10.1	2.3
	FA	0.6	4.4	33.2	9.1	2.0	4.5	31.1	11.8	4.0
	TG	92.8	1.7	23.9	10.0	1.9	4.6	42.7	14.7	0.2
Mature seed	PL + GL	2.9	11.7	47.7	23.1	2.9	1.4	5.1	4.4	3.7
	MG	0.1	12.5	33.2	12.2	3.3	2.5	18.7	10.2	7.4
	DG	0.4	3.9	32.3	9.4	0.8	1.6	31.6	16.0	4.4
	FA	0.3	5.2	26.0	5.9	1.0	1.3	35.7	18.4	6.5
	TG	96.3	1.1	22.7	5.5	0.7	1.8	42.2	25.8	0.2

*PL, Phospholipids; GL, glycolipids; MG, monoacylglycerols; DG, diacylglycerols; FA, unesterified fatty acids; TG, triacylglycerols.

†See footnote, Table 1.

‡Including 16:1, 18:0, 20:0, 22:0 and 24:0.

Table 3. Composition of the major classes of phospholipids and glycolipids in seeds of *L. annua* 6–7 weeks after flowering

Lipid class*	Fatty acid† composition (wt %)					
	16:0	18:1	18:2	18:3	20:1–24:1	Others‡
PC	15.1	39.1	28.1	11.3	3.5	2.9
PE + PG	17.6	26.3	34.5	12.9	4.6	4.1
PA	13.6	34.9	32.4	13.3	3.7	2.1
MGDG	4.0	17.8	28.4	40.4	6.7	2.7
DGDG	22.9	17.6	25.5	27.6	3.1	3.3

*PC, Phosphatidylcholines; PE, phosphatidylethanolamines; PG, phosphatidylglycerols; PA, phosphatidic acids; MGDG, monogalactosyldiacylglycerols; DGDG, digalactosyldiacylglycerols.

†See footnote, Table 1.

‡Including 16:1, 18:0, 20:0, 22:0 and 24:0.

Table 4. Composition of the predominant fraction of triacylglycerols in seeds of *L. annua*

Sample	Acyl moieties*	% of total triacylglycerol species	Acyl† composition (wt %)					
			18:1	18:2	20:1	22:1	24:1	Others‡
Developing seed (I)†	Total	81.7	28.8	11.5	5.0	39.0	12.4	3.3
	sn-2		67.2	31.2	0.5	0.5	tr	0.6
	sn-1,3		9.6	1.6	7.2	58.2	18.6	
Developing seed (II)†	Total	90.6	25.5	8.7	4.5	45.0	14.2	2.1
	sn-2		69.0	29.5	0.5	0.4	tr	0.5
	sn-1,3		3.7	−1.7	6.5	67.3	21.3	
Mature seed	Total	97.9	23.3	6.1	0.7	41.7	25.9	2.3
	sn-2		79.2	8.7	0.4	3.5	2.8	5.4
	sn-1,3		−4.6	4.8	0.8	60.7	37.4	

*Composition of the acyl moieties at the *sn*-2 position was determined by hydrolysis with pancreatic lipase; composition of the acyl moieties at the *sn*-1,3 positions was then calculated.

†See footnote, Table 1.

‡Including 16:0, 16:1, 18:0, 18:3, 20:0, 22:0 and 24:0.

insignificant proportions of very long chain monounsaturated acyl moieties. The preponderance of very long chain monounsaturated acyl moieties in triacylglycerols and other neutral lipids, but not in the phospholipids and glycolipids, has been documented for other cruciferous seeds [6, 10].

The triacylglycerols isolated from each of the developing and mature seeds have been fractionated by argentation TLC into fractions differing in their degree of unsaturation. The composition of the major fractions (Table 4) shows that the most predominant fraction of triacylglycerols in the mature seed is almost exclusively composed of 24:1, 22:1 and 18:1 moieties. The relative proportion of the triacylglycerol fraction containing these monounsaturated acyl moieties is found to increase with progressive maturation of *L. annua* seed. The minor fractions of triacylglycerols detected in both developing and mature seeds include the types 'monoenoyl-dienoyl-monoenoyl' and 'monoenoyl-trienoyl-monoenoyl' (data not shown). Lipolysis of the major fraction of triacylglycerols with pancreatic lipase revealed that 24:1 and 22:1 moieties are almost exclusively located at the *sn*-1,3 positions of glycerol, whereas the 18:1 moieties are confined to the *sn*-2 position (Table 4). Preponderance of 22:1 and 20:1 moieties at the *sn*-1,3 positions of triacylglycerols has also been documented for the seeds of rape [17] and crambé [8], and this seems to be a common feature of triacylglycerols of most cruciferous seeds.

Although the pathway for the biosynthesis of very long chain (n − 9)monounsaturated fatty acids in higher plants is well established [11–16], the mechanism of formation of triacylglycerols containing such very long chain acyl moieties is not yet fully understood [10, 16, 18, 19]. Developing seeds of *L. annua*, which actively synthesize 'only one' major fraction of triacylglycerols containing the very long chain acyl moieties at the *sn*-1,3 positions, might provide a suitable model for studying the biosynthesis of such triacylglycerols.

EXPERIMENTAL

The plants of *L. annua*, garden variety, were field-grown and the seeds collected at defined intervals after flowering.

Procedures used for the extraction of lipids and their fractionation, isolation as well as identification by TLC were identical to those described previously [10]. In order to determine the acyl composition of lipids and the rel. proportions of the individual lipid classes in the total lipids, each lipid fraction was transmethylated and the Me esters analysed by FID-GC, as described elsewhere [4], using Me 17:0 as int. standard [20].

Me esters of total lipids were fractionated by $AgNO_3$ TLC and prep. GC into 18:1, 20:1, 22:1 and 24:1; subsequently, the position of the olefinic bond in each Me ester fraction was determined by reductive ozonolysis and GC of the resulting fragments [4].

Triacylglycerols isolated from the total lipids were fractionated by TLC on silica gel G containing 20% $AgNO_3$ with $CHCl_3$–MeOH (19.9:0.1) into various fractions, which were located under UV after spraying with 0.2% ethanolic 2',7'-dichlorofluorescein and isolated by eluting with H_2O-satd Et_2O. The rel. proportions of various triacylglycerol fractions and their acyl composition were determined by transmethylation of an aliquot and subsequent GC analysis of the Me esters using Me 17:0 as int. standard, as described above. The composition of acyl moieties at the *sn*-2 position of the triacylglycerols was determined by hydrolysis with porcine pancreatic lipase, followed by isolation of the 2-acylglycerols [21], which were converted to Me esters and analysed by GC [4]. The composition of acyl moieties at the *sn*-1,3 positions was calculated according to ref. [21].

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