Phytochemistry, 1976, Vol. 15, pp. 1727–1729. Pergamon Press. Printed in England.

LOCALIZATION OF LIPIDS CONTAINING (Z)-11-EICOSENOIC ACID AND (Z)-13-DOCOSENOIC ACID IN *TROPAEOLUM MAJUS*

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(*Revised received* 29 *April* 1976)

Key Word Index—*Tropaeolum majus*; Tropaeolaceae; nasturtium; lipids; unusual fatty acids; distribution.

Abstract—(Z)-11-Eicosenoic (gondoic) and (Z)-13-docosenoic (erucic) acids were found in large proportions as constituent fatty acids of the triglycerides and polar lipids in seeds and petals of *Tropaeolum majus*. In the lipids of the other floral organs as well as in those of vegetative organs, only traces of these fatty acids were detected. During seed germination, the proportions of the two fatty acids did not change. (Z)-9,12-Octadecadienoic (linoleic) and (Z)-9,12,15-octadecatrienoic (linolenic) acids, which occurred only in traces in lipids of the seeds, were major constituent fatty acids of lipids in floral and vegetative organs as well as those of callus cultures.

INTRODUCTION

In studies on the lipids of higher plants much effort has been devoted to seeds, as sources of oil, and to green leaves, in order to investigate the possible role of certain lipid classes in photosynthesis [1]. The lipids and their constituent fatty acids in vegetative organs, such as the roots, stems, leaf blades and petioles, and in the various floral organs have not received sufficient attention. Less common fatty acids such as (Z)-11-eicosenoic (gondoic) and (Z)-13-docosenoic (erucic) acids, which are known to predominate as constituents of the lipids in seeds of plants belonging to the family Cruciferae are found only in traces in the growing axes of seedlings of these plants [2]. Data on the distribution of these fatty acids in the other organs of mature plants are not available.

The seed lipids of *Tropaeolum majus* are characterized by high levels of (Z)-11-eicosenoic and (Z)-13-docosenoic acids [3,4] and the virtual absence of polyenoic fatty acids which usually occur as constituents of the lipids in seeds of higher plants.

The present investigation is concerned with the systematic analysis of the constituent fatty acids of lipids in seeds and all organs of *T. majus* as well as in callus cultures derived from this plant. Both vegetative and floral organs were studied in an attempt to localize the sites where the less common constituent fatty acids are synthesized. An additional objective was to study the distribution of polyunsaturated fatty acids in the lipids from different organs and callus cultures of *T. majus*.

RESULTS

Lipid contents

The seeds, vegetative organs, flower stalks, and floral organs as well as callus cultures of *T. majus* differed in their lipid contents, the highest values being those of the floral organs (Table 1). Thus, in the sepals, stamens and gynaesia the lipids constituted about one third of the organs' dry wt. In comparing the data given in Table 1 it should be noted that the extracts of the samples analyzed, except those of seeds, roots and callus cultures, contained appreciable proportions of chlorophylls, carotenoids and other pigments.

Composition of lipid classes

Triglycerides were the major lipid class only in seeds, where the ionic and other polar lipids were present in very small proportions. In contrast, the extracts from the vegetative and floral organs as well as those from the callus cultures contained predominantly ionic and other polar lipids. These included phosphatidylcholines and, to a lesser extent, phosphatidylethanolamines, steryl glycosides, and esterified steryl glycosides. In green organs, such as the leaf blades, monogalactosyldiglycerides, digalactosyldiglycerides and sulfoquinovosyldiglycerides were abundant; these compounds were detected only in traces, if at all, in seeds, roots, stamens, gynaesia and callus cultures. In all the samples analyzed, sterols

Table 1. Lipid contents of seeds, organs and callus cultures of *T. majus**

Organ	Total lipids %
Seeds	9.6
Vegetative organs:	
Roots	12.4
Stems	9.1
Leaf blades	10.5
Leaf petioles	13.9
Flower stalks	26.5
Floral organs:	
Sepals	36.2
Petals	23.3
Stamens	35.1
Gynaesia	35.8
Callus cultures	8.1

* Data are expressed as % of dry wt.

Table 2. Fatty acid patterns of total lipids from seeds, organs and callus cultures of *T. majus**

Fatty acid	Vegetative organs					Floral organs				Callus cultures
	Seeds	Roots	Stems	Leaf blades	Leaf petioles	Flower stalks	Sepals	Petals	Stamens	Gynaesia	
14:0	tr	tr	tr	tr	tr	tr	4.0	2.0	tr	tr	tr
16:0	2.4	27.9	26.1	32.0	27.1	32.0	27.2	11.3	24.4	28.3	27.1
18:0	—	4.8	4.3	1.3	2.4	4.0	9.0	4.2	2.3	8.3	tr
18:1	10.3	11.8	7.2	3.5	4.8	4.0	4.1	10.7	7.7	11.7	35.3
18:2	tr	41.4	48.0	23.2	46.7	44.0	22.5	17.6	17.8	26.7	14.6
18:3	tr	14.1	14.4	40.0	19.0	16.0	33.2	14.1	47.8	25.0	23.0
20:1	24.8	tr	tr	tr	tr	tr	tr	5.6	tr	tr	tr
22:1	62.5	tr	tr	tr	tr	tr	tr	34.5	tr	tr	tr

* Data are expressed as wt %; tr = trace, — = not detected.

and steryl esters as well as hydrocarbons were found in appreciable proportions.

It was found that during germination of *T. majus* seeds the concentration of triglycerides did not change whereas the proportions of phospholipids increased.

Fatty acid patterns

(Z)-13-Docosenoic acid was the major constituent fatty acid in the lipids of the seeds and petals; (Z)-11-eicosenoic acid was detected in appreciable amounts also in the lipids of seeds and petals (Table 2). In other organs and in callus cultures these two fatty acids were present in trace amounts. Palmitic acid was relatively low in the seed lipids whereas in the lipids of the various organs as well as in the callus cultures it was one of the major constituent fatty acids. Stearic acid was not detected in the seed lipids but occurred in considerable proportions in the lipids of the organs and, in traces, in callus cultures. Oleic acid predominated in the lipids of callus cultures, linoleic acid in the lipids of the roots, stems, petioles and flower stalks, and linolenic acid was predominant in the lipids of the leaf blades, stamens and sepals. In the gynaesiae nearly equal proportions of linoleic and linolenic acids were detected.

In the seeds and petals eicosenoic and docosenoic acids were esterified in polar lipids as well as in nonpolar lipids, though at different levels (Table 3).

During germination of *T. majus* seeds, the proportions of eicosenoic and docosenoic acids in the total lipids remained essentially constant whereas the concentrations of palmitic, linoleic and linolenic acids increased and those of oleic acid decreased (Table 4).

Table 3. Fatty acid patterns of polar and nonpolar lipid fractions from seeds and petals of *T. majus**

Fatty acid	Seeds		Petals	
	Polar lipids	Nonpolar lipids	Polar lipids	Nonpolar lipids
14:0	tr	tr	6.3	2.7
16:0	21.4	1.5	12.5	10.0
18:0	8.6	—	3.1	4.5
18:1	20.0	7.5	7.1	13.7
18:2	1.3	tr	11.3	10.9
18:3	tr	tr	32.5	4.5
20:1	10.7	25.0	4.2	10.0
22:1	38.0	66.0	23.0	43.7

* Data are expressed as wt %; tr = trace, — = not detected.

Seedlings incubated at 5° contained more linolenic acid than those maintained at 30° (Table 4).

DISCUSSION

It is surprising that in all floral organs of *T. majus* the total lipids constitute about one third of the organs' dry wt whereas the seeds contain less than 10% lipids. Even the flower stalks are much richer in lipids than the seeds.

The composition of the lipid classes in the floral organs is similar to that in the vegetative organs although the latter contain higher proportions of monogalactosyldiglycerides, digalactosyldiglycerides and sulfoquinovosyldiglycerides. Such lipid classes are present especially in the leaf blades.

It is interesting to note that in *T. majus* eicosenoic and docosenoic acids occur not only in the seed lipids but also in the lipids of the petals, both in the polar and nonpolar fractions. However the gynaesia, where the seeds develop, contain neither eicosenoic nor docosenoic acids as lipid constituents.

The concentrations of eicosenoic and docosenoic acids in the seed lipids remain constant during germination. These fatty acids are localized in the cotyledons [2] which eventually wither in the soil. Of interest is the fact that the polyunsaturated fatty acids that occur only in traces in the seed lipids, namely linoleic and linolenic acids, are major constituent fatty acids of the lipids of various organs of the mature plants. Callus cultures of *T. majus* do not synthesize eicosenoic and docosenoic acids in appreciable proportions thus resembling cultures of *Brassica napus* [2]. Similar to the lipids in the various

Table 4. Fatty acid patterns of seeds and seedlings of *T. majus* incubated at 5° and 30°*

Fatty acid	Seeds	Seedlings	
		5°	30°
14:0	tr	tr	tr
16:0	2.4	3.7	3.9
18:0	—	tr	tr
18:1	10.3	6.5	8.7
18:2	tr	2.4	3.9
18:3	tr	2.5	0.4
20:1	24.8	21.6	22.2
22:1	62.5	63.3	60.9

* Data are expressed as wt %; tr = trace, — = not detected.

organs, the lipids in callus cultures contain large proportions of linoleic and linolenic acids.

In view of the results presented it appears possible that unusual fatty acids, such as hydnocarpic and chaulmoogric acids, which predominate as constituent fatty acids in the seed lipids [5] but not in the lipids of leaves [6] and callus cultures [7] of certain plants, are also synthesized in the petals of these plants. Thus, petals may be suitable for studying the biosynthesis and metabolism of less common and unusual fatty acids in higher plants.

EXPERIMENTAL

Mature seeds as well as the vegetative and floral organs of *Tropaeolum majus* were collected from plants cultivated in the garden. Seedlings were obtained by germinating seeds at 30° for 7 days. Some seedlings were incubated at 5° and others at 30° for another 7 days. Callus cultures were established from seedlings using the nutrient medium and the experimental conditions described previously [8].

Lipids were extracted and purified following established procedures [9,10]. The classes of nonpolar lipids were resolved by TLC on Si gel with hexane–Et_2O–HOAc (90:10:1) [11] and the ionic and other polar lipids with $CHCl_3$–MeOH–H_2O (65:25:4) [12] and $CHCl_3$–MeOH–HOAc (85:15:1) [9]. Lipid classes were identified by comparing their R_f values with those of reference samples and by their reactions with specific spray reagents [13,14].

Aliquots of the total lipids and aliquots of the fraction of non-polar lipids as well as of the fraction of ionic and other polar lipids were subjected to methanolysis [15]. The resulting Me esters were purified by TLC [15] and analyzed by GLC on a polar (15% DEGS on Anakrom D) and a non-polar column (10% Silar 5 CP on Gas-Chrom Q).

Acknowledgement—Financial support by the Alexander von Humboldt-Foundation is gratefully acknowledged.

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