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The Lipid Composition of Fresh *Origanum dictamnus* Leaves

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ABSTRACT

The components of the lipid fraction of fresh leaves of Origanum dictamnus *L. have been identified by means of chromatographic techniques. The non-polar lipids identified were: sterols, steryl esters, free fatty acids, fatty alcohols, triglycerides, waxes, hydrocarbons, carvacrol, esters and triterpenic acids. The following polar lipids were also identified: mono-, di-, and polygalactosyl diglycerides, sulpholipids, cerebrosides, phosphatidyl-ethanolamine, phosphatidyl-serine, phosphatidyl-glycerol, phosphatidyl-inositol and phosphatidyl-choline. No phosphatidic acid was detected.*

INTRODUCTION

Origanum dictamnus L. is one of the best known pharmaceutical plants. Several therapeutic properties have been attributed to substances present in the lipoidic extract of plants (Kavvadas, 1956), e.g. sterols (Sapeica, 1969) and polyphenols (Theodossiou, 1972).

Although it has been known and used as a herb tea for many years its chemical composition has not been thoroughly investigated. In 1972, Theodossiou reported the presence of polyphenols. Revinti-Moraiti *et al.* (1985) reported the lipid composition of dried *Origanum dictamnus* leaves.

The purpose of this work, which is a continuation of previous studies, is to determine the lipid composition of fresh leaves of this particular plant and investigate the presence of lipoidic compounds possessing therapeutic properties.

MATERIALS AND METHODS

Materials

The *Origanum dictamnus* leaves were collected from Crete, Greece. All the reagents used were of analytical grade. Cerebrosides and sulpholipids were obtained from spinach leaves and rat brain, respectively.

Extraction

A quantity (2 g) of fresh leaves was heated over a steam bath for 10 min (Yang *et al.*, 1967). Under these conditions the phospholipase activity is slight. After this treatment the leaves were homogenised and extracted with 38 ml of chloroform (2:1) for 2 min. After filtration the residue was washed with chloroform–methanol (2:1 v/v). The combined filtrates were treated according to the procedure of Folch *et al.* (1957). The chloroformic phase, which contained the total lipids, was taken to dryness in a rotary evaporator. The isolated lipids were stored at 0°C.

Separation and identification

The lipid extract was subjected to column chromatography using a silicic acid (Bio-Sil HA-325 mesh, Biorad) column. The elution system used was similar to that described by Rouser *et al.* (1967*a*). The lipids were separated into three classes: non-polar lipids (chloroformic eluate), glycolipids (acetonic eluate) and phospholipids (methanolic eluate).

Each eluate was checked with thin-layer chromatography (TLC) which was performed on 20 × 20 cm or 5 × 10 cm chromatoplates covered with silica gel G (0·25–0·50 mm thickness). The elution systems used were as follows.

Non-polar lipids

(1) Petroleum ether (60–80°C):diethyl ether:acetic acid (80:20:1 v/v), (70:30:2 v/v) for all non-polar lipids (Mangold, 1969).
(2) Chloroform:acetone (9:1 v/v) for triterpenic acids (O.J.E.C., 1977).
(3) Hexane:acetone (85:15 v/v) for the unsaponified fraction (O.J.E.C., 1977).
(4) Chloroform:acetone:methanol:acetic acid (73:25:15:0·5 v/v) for triterpenic acids (Clayton *et al.*, 1970).
(5) Toluene:acetone:acetic acid (100:3:0·07 v/v) for triterpenic acids (Mangold, 1969).

Polar lipids

Solvent systems used for one-dimensional chromatography:

(1) Chloroform:methanol:water (65:25:4 v/v) for glyco- and phospholipids (Lepage, 1964).
(2) Chloroform:acetone:methanol:acetic acid:water (6:8:2:2:1 v/v) for phospholipids (Rouser *et al.*, 1969).
(3) Ethyl acetate:*n*-propanol:chloroform:methanol:0·25% aqueous solution of potassium chloride (25:25:25:10:9 v/v) for separation of glyco- and phospholipids (Vitielo & Zanetta, 1978).
(4) Acetone:benzene:water (91:30:8 v/v) for glycolipids (Pohl *et al.*, 1970).
(5) Di-isobutyl ketone:acetic acid:water (80:50:10 v/v) for phospholipids (Lepage, 1964).

Solvent systems used for two-dimensional chromatography:

(1) a. Chloroform:methanol:ammonia (7N) (65:35:4 v/v).
b. Chloroform:methanol:acetic acid:water (170:25:25:6 v/v) for separation of polar lipids (Nickols, 1964).
(2) a. Chloroform:methanol:water (65:25:4 v/v).
b. Butanol:acetic acid:water (6:2:2 v/v) for polar lipids (Rouser *et al.*, 1967*b*).

The visualisation of the lipids was effected by spraying with specific reagents:

(1) Sulphuric acid 50% and heating to 110°C (general purpose reagent).
(2) 0·01% w/v rhodamine 6G for all lipids (Marinetti, 1964).
(3) Copper acetate:phosphoric acid and heating at 180°C for 25 min for polar and non-polar lipids (Fewster *et al.*, 1969).
(4) 0·2% anthrone in concentrated sulphuric acid for sulpho- and galactolipids (Galliard, 1968).
(5) The Dittmer–Lester reagent for phospholipids (Dittmer & Lester, 1964).
(6) 0·2% w/v ninhydrin reagent in ethanol for lipids with a free amino group (Marinetti, 1964).
(7) 0·2% w/v orcinol reagent in a solution of sulphuric acid and water (3:1) for detection of glycolipids (Svenerholm, 1956).
(8) 0·2% 2,7-dichlorofluorescein in ethanol (95°) for detection of neutral lipids (Kates, 1972).
(9) *a*-naphthol reagent for glycolipid detection (Jacin and Mishkin, 1965).
(10) Lieberman–Burchard reagent and antimony (III) chloride (20% w/v

in chloroform) for detection of sterols and triterpenic acids (Takeda *et al.*, 1963).

(11) Dragendorff reagent for detection of phospholipids containing choline (Beiss, 1964).

Gas chromatographic analysis

The fatty acid methyl esters were prepared by treatment of the lipids with boron–trifluoride methanol (Morrison & Smith, 1964). Methyl esters were purified by preparative TLC.

Qualitative and quantitative determinations were performed by gas–liquid chromatography with a Tracor (model 222) gas chromatograph equipped with a flame ionisation detector. The columns used were Carbowax 20 M 5% on Chromosorb W (60–80 mesh) and OV-1 3% on 60–80 mesh Chromosorb W. The flow rate of the carrier gas (nitrogen) was 60 ml min^{-1} and the columns were operated isothermally at 196°C.

Quantitative determination of lipids

Total non-polar and polar lipids were determined by weight. The organic phosphate (Barlette, 1959) and the sugar content (Roughan & Batt, 1968) were also assessed.

RESULTS AND DISCUSSION

The lipid composition of fresh *Origanum dictamnus* leaves is shown in Table 1. The results were calculated on a dry basis.

Polar lipids separated by TLC in the aforementioned solvent systems were identified by comparison with standard compounds.

The glycolipids identified were:
Monogalactosyl-diglycerides (MDGD), digalactosyl-diglycerides (DGDG), sulpholipids (SL), cerebrosides (CER), and polygalactosyl-diglycerides (PGDG).

The glycolipids represented 84·4% of the polar lipids and 27% of the total lipids. The presence of the PGDG was verified on TLC by employment of standard compounds isolated from spinach leaves. The quantitative determination was based on the sugar content. Galactose was the standard compound and the calculation of the glycolipids was based on empirical factors (Hansen, 1975; Leese & Leech, 1976). The dominant glycolipid was the MDGD (37·5% of the polar lipids). It contained a high proportion of linoleic acid. In the glycolipids of the dried leaves, the SL and CER were the

TABLE 1
Quantitative Composition of Lipids of Fresh *Origanum dictamnus* Leaves

Lipid	*% w/w of fresh leaves*[b]	*% w/w of total lipids*	*% w/w of polar lipids*	*% w/w of glyco-lipids*	*% w/w of phospho-lipids*
Total lipids	20·0				
Non-polar lipids	13·6	68·0			
Polar lipids	6·38	32·0			
Glycolipids	5·38	27·0	84·4		
Phospholipids	1·00	5·00	15·6		
MDGD	2·39	12·0	37·5	44·4	
DGDG	1·24	6·20	19·4	23·0	
CER	0·90	4·50	14·0	16·7	
SL	0·76	3·80	12·0	14·0	
PDGD[a]	0·09	0·50	1·50	1·90	
PE	0·14	0·70	2·10		14·4
PG	0·24	1·20	3·80		23·3
PI	0·09	0·45	1·40		9·00
PS	0·19	0·95	3·00		17·8
PC	0·34	1·70	5·30		35·5

[a] Assume 3 moles hexose per mole of lipid.
[b] Mean of three determinations.

major constituents and, as expected, the percentage of linoleic acid was very low (Revinthi-Moraiti *et al.*, 1985).

The sugar identified in the cerebroside fraction was glucose, while the other glycolipids contained galactose.

Palmitic, oleic and linoleic acids were the most abundant fatty acids in the DGDG, SL and CER (Table 2).

The non-polar lipids represented 68% of the total lipids and the identified compounds were: Sterols, steryl esters, fatty alcohols, free fatty acids, traces of triglycerides, waxes, carvacrol (known for its pharmaceutical properties) and triterpenic acids. The latter showed the same R_f as ursolic acid and gave a violet colour upon spraying with the Brieskorn–Briner reagent (Brieskorn *et al.*, 1954). Quantitative determination showed that they constituted 14·5% of the total lipids. The main fatty acids of the non-polar fraction were: palmitic, oleic and linoleic (Table 2).

The phospholipids were present in small quantities (1% w/w). They constituted 5% of the total lipids and 15·6% of the polar lipids. These percentages are higher than those reported for dried leaves (Reventhi-Moraiti *et al.*, 1985). The quantitative determination of phospholipids was based on the determination of organic phosphate (Barlette, 1959).

TABLE 2
Fatty Acid Composition of *Origanum dictamnus* Lipids[a]

Lipid	*Fatty acids (relative area %)*[b]							
	14:0	*16:0*	*16:1*	*16:3*	*18:0*	*18:1*	*18:2*	*18:3*
Non-polar lipids	3·0	47·2	1·0	—	6·5	18·0	16·5	7·6
Free fatty acids	1·8	48·0	5·6	—	3·2	19·0	14·8	7·6
Triglycerides	3·0	50·0	1·0	—	2·5	19·2	10·9	13·4
MDGD	Trace	3·3	0·6	0·9	0·8	2·3	3·2	89·0
CER	1·3	33·2	4·5	0·7	4·3	21·0	29·2	5·8
DGDG	8·0	31·5	2·7	1·0	1·2	26·8	26·5	2·3
SL	Trace	39·2	5·0	Trace	1·0	30·4	22·4	2·0
PE	1·5	18·7	2·3	4·0	5·0	24·3	29·0	15·2
PG	Trace	17·0	6·0	4·7	3·0	16·7	11·1	41·5
PI	Trace	31·0	Trace	Trace	4·0	30·5	23·7	10·8
PS	4·5	28·0	1·6	—	3·0	32·0	24·8	6·1
PC	Trace	53·5	Trace	—	1·0	11·0	19·4	15·1

[a] Only 0·5% or more are reported.
[b] Means of six determinations.

The phospholipids identified were: Phosphatidyl-ethanolamine (PE), phosphatidyl-glycerol (PG), phosphatidyl-inositol (PI), phosphatidyl-choline (PC) and phosphatidyl-serine (PS). The existence of such phospholipids is in agreement with the findings of other workers (Kates, 1960*a*; Wintermans, 1963). No phosphatidic acid was detected. This was attributed to steam heating which prevented any enzymic hydrolysis of the phospholipids (Kates, 1960*b*; Haverkate & Van Deenen, 1965; Yang *et al.*, 1967).

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