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# Identification and Quantitative Determination of the Lipids of Dried *Origanum dictamnus* Leaves

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*ABSTRACT*

*The* Origanum dictamnus *plant was examined for its lipid and fatty acid compositions. A combination of chromatographic techniques has been employed for the qualitative and quantitative determination of the lipids of dried leaves of* O. dictamnus. *The following polar lipids were identified: mono-, di- and poly-digalactosyl diglycerides, sulpholipids, cerebrosides, phosphatidyl-ethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, phosphatidyl choline and phosphatidic acid. The non-polar lipids identified were sterols, steryl esters, fatty alcohols, free fatty acid, waxes, traces of triglyceride, triterpenic acids and essential oil. The predominant fatty acids were palmitic, oleic and linoleic acids.*

## INTRODUCTION

*Origanum dictamnus* L. is one of the most well-known herb teas and it has been extensively used for its therapeutic properties. Several pharmaceutical properties have been attributed to substances present in the lipoid extracts of plants (Kavvadas, 1956), e.g. sterols (Sapeica, 1969) and polyphenols (Theodossiou, 1972).

The lipids of *O. dictamnus* have not been investigated and the present work is a detailed study of the lipid and fatty acid compositions of this plant. The presence of substances possessing pharmaceutical properties



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(other than polyphenols, already investigated by Theodossiou (1972)) is also under consideration.

## MATERIALS AND METHODS

The *O. dictamnus* plants used for this study were grown in Crete. Lipids which were used as standards were from commercial sources except cerebrosides and sulpholipids which were obtained from spinach leaves and rat brain.

### Extraction

Dried leaves (1 g) were minced and homogenised with 19 ml of chloroform–methanol (2:1 v/v) for 2 min. The homogenate was filtered under suction and the residue was rehomogenised with the same volume of solvent. 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 final chloroform phase which contained the total lipid of the dry leaves was taken to dryness in a rotary evaporator at 40 °C. The isolated lipids were dissolved in a chloroform–methanol solution (2:1 v/v) and stored at 0 °C (Kates, 1972).

### Column chromatography

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

### Thin-layer chromatography (TLC)

Thin-layer chromatography was performed on 20 × 20 cm or 5 × 10 cm chromatoplates covered with silica gel G (0·25–0·50 mm thickness). The solvent systems used were:

*Non-polar lipids*

(1) Petroleum ether (60–80 °C) diethylether–acetic acid (80:20:1 v/v), (70:30:2 v/v/v) (Mangold, 1969) for all non-polar lipids.

(2) Chloroform–acetone (90:10 v/v) (O.J.E.C.) for triterpenic acids.
(3) Hexane–acetone (85:15 v/v) (O.J.E.C.) for the unsaponified fraction.
(4) Chloroform–acetone–methanol–acetic acid (73:25:15:0·5 v/v) (Clayton *et al.*, 1970) for triterpenic acids.
(5) Toluene–acetone–acetic acid (100:3:0·07 v/v) (Mangold, 1969) for triterpenic acids.

*Polar lipids*

The solvent systems used for one-dimensional chromatography were:

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

For the two-dimensional chromatography the solvent systems were:

(1) a. Chloroform–methanol–ammonia 7N (65:35:4 v/v).
    b. Chloroform–methanol–acetic acid–water (170:25:25:6 v/v) (Nickols, 1964) for separation of polar lipids (MGDG, CER, PG, PE, DGDG, SL, PC, PI, PS).
(2) a. Chloroform–methanol–water (65:25:4 v/v)
    b. Butanol–acetic acid–water (6:2:2 v/v) (Rouser *et al.*, 1967*b*) for separation of polar lipids.

The identification of sugars was effected after development in a solvent system consisting of iso-propanol–acetone–0·1 M lactic acid (4:4:2 v/v) on silica gel G and subsequent spray with a specific reagent.

Spots of separated lipid materials were initially detected on analytical plates with iodine vapour followed by specific spray reagents:

(1) Sulphuric acid 50% and heating to 110 °C (general-purpose reagent).

(2) Copper acetate–phosphoric acid and heating for 25 min at 180 °C (Fewster *et al.*, 1969) for detection of polar and non-polar lipids.
(3) 0·01 % w/v rhodamine 6G (Marinetti, 1964) for all lipids.
(4) 0·2 % w/v anthrone in concentrated sulphuric acid (Galliard, 1968) for galacto- and sulpho-lipids.
(5) The Dittmer–Lester (Dittmer & Lester, 1964) reagent for phospholipids.
(6) 0·2 % w/v orcinol reagent in a solution of sulphuric acid–water (3:1) for the detection of glycolipids.
(7) 0·2 % w/v ninhydrin reagent in ethanol for lipids with a free amino group.
(8) 0·2 % w/v 2,7-dichlorofluorescein in ethanol (95 °) for detection of neutral lipids.
(9) α-Naphthol reagent (Jacin & Mishkin, 1965) for glycolipid detection.
(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–liquid chromatography

The methyl esters of fatty acids were prepared according to the method of Morrison & Smith (1964). Methyl esters were purified by preparative TLC.

A Tracor (model 222) gas chromatograph, equipped with a flame ionisation detector, was used for the analysis of methyl esters. The columns used were Carbowax 20M 5 % on 60–80 mesh Chromosorb W, and OV-1 3 % on 60–80 mesh Chromosorb. The columns were operated isothermally at 195 °C. The carrier gas used was nitrogen (flow rate 60 ml/min).

Fatty acids were identified by comparison of their retention times with those of pure compounds.

## Quantitative analysis of lipids

The eluates collected from the silicic acid column were separated by preparative TLC and each component was studied separately. The

organic phosphate (Barlette, 1959) and the sugar content (Roughan & Batt, 1968; Leese & Leech, 1976) were also assessed.

Total non-polar and polar lipids were determined by weight.

## RESULTS AND DISCUSSION

The total lipid (TL) content of dried leaves of *O. dictamnus* was found to be 9·72% on a dry basis. The dried leaves contained 81% of non-polar lipids and 19·0% polar lipids. Glycolipids represented approximately 90% of the polar lipids (Allen *et al.*, 1963; Smith & Wolff, 1966; Roughan & Batt, 1968, 1969; Hitchcock & Nickols, 1971).

### Polar lipids (PL)

The polar lipids were identified by TLC in the above-mentioned solvent systems with known standards and use of specific spraying reagents for detection. The identified glycolipids were monogalactosyl-diglycerides (MGDG), digalactosyl-diglycerides (DGDG), sulpholipids (SL), cerebrosides (CER) and polygalactosyl diglycerides (PGDG) (Svennerholm, 1956; Galliard, 1968).

### Cerebroside

Spot 3 gave a dull purple spot on TLC when sprayed with 50% sulphuric acid and heated at 110°C. Acid hydrolysis resulted in a base exhibiting similar properties on TLC to sphingosine, glucose and free fatty acids (Wagner *et al.*, 1961; Sastry & Kates, 1964; Fedelli & Jacini, 1971; Vitielo & Zanetta, 1978). Spots 4 and 2 were found to be MGDG and DGDG, respectively. Spot 1 gave the characteristic violet colour of sulpholipids on TLC when sprayed with an anthrone solution, while the other glycolipids gave green spots.

Spot 0 had the same $R_f$ as polygalactolipids (PGDG) (Figs 1(a) and 1(b)). The presence of polygalactolipids was verified on TLC by the employment of standard lipids isolated from spinach leaves.

In each glycolipid, the sugar moieties (Hansen, 1975) and the fatty acid contents (Morrison & Smith, 1964) were examined (Table 1). Quantitative determination was based on the sugars present. Galactose was the standard

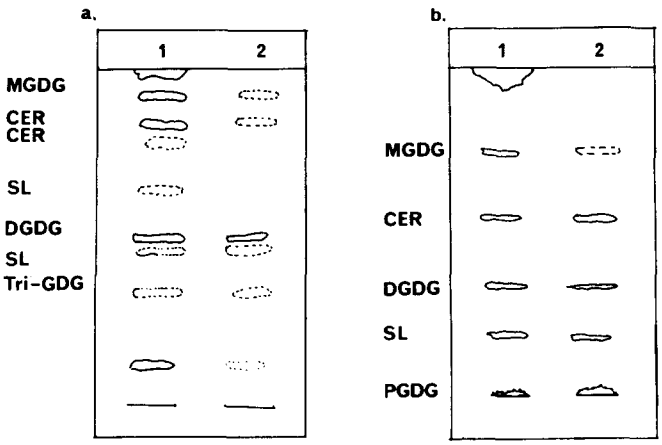


**Fig. 1.** Thin-layer chromatogram of glycolipids of *O. dictamnus* leaves. (a) Solvent system: ethyl acetate–*n*-propanol–chloroform–methanol–0·2% aqueous solution of potassium chloride (25:25:25:10:9 v/v). Visualisation (0·2%) orcinol solution and heating (100 °C). (b) Solvent system: acetone–benzene–water (90:30:8 v/v). Visualisation: (0·2%) orcinol solution and heating or 0·2% anthrone solution. 1—Lipoidic extract of spinach leaves; 2—fraction of polar lipids of dried *O. dictamnus* leaves.

compound and the calculation of the glycolipids was based on empirical factors (Leese & Leech, 1976).

MGDG contained galactose and the fatty acid found in abundance was palmitic (50·6%). MGDG represented 17·4% of the polar lipids and 19% of the glycolipids. Sulpholipids were the dominant glycolipids (97·4% of glycolipids) (Roughan & Batt, 1968). Palmitic (36·6%) and linoleic (31·6%) acids were found in higher proportions in DGDG.

The sugar identified in the cerebroside fraction was glucose while palmitic (40·7%), oleic (29·1%) and linoleic (17·7%) acids were the most abundant fatty acids.

The sugar identified in the sulpholipid was galactose and the predominant fatty acids were palmitic (48%), oleic (25·1%) and linoleic (19·1%) acids.

## Phospholipids

The existence of such phospholipids is in agreement with previous findings (Kates, 1960*a*; Wintermans, 1963). The well-identified phospholipids were PC, PE, PG, PI and PS. The identification was effected on TLC in at least two solvent systems. The minor constituent was

**TABLE 1**
Quantitative Composition of *O. dictamnus* Lipids

| *Lipid* | % *w/w of dried leaves* | % *w/w of total lipids* | % *w/w of polar lipids* | % *w/w of glyco-lipids* | % *w/w of phospho-lipids* |
|---|---|---|---|---|---|
| Total lipids | 9·72 | | | | |
| Non-polar lipids | 7·86 | 81·05 | | | |
| Polar lipids | 1·85 | 18·95 | | | |
| Glycolipids | 1·66 | 17·00 | 89·85 | | |
| Phospholipids | 0·20 | 1·95 | 10·15 | | |
| MDGD | 0·35 | 3·45 | 17·40 | 19·7 | |
| DGDG | 0·25 | 2·60 | 13·7 | 15·3 | |
| CER | 0·42 | 4·35 | 23·5 | 26·1 | |
| SL | 0·46 | 4·65 | 24·7 | 27·4 | |
| PGDG[a] | 0·18 | 1·95 | 10·7 | 11·7 | |
| PA[b] | 0·009 | 0·10 | 0·55 | | 5·55 |
| PE[b] | 0·033 | 0·31 | 1·63 | | 16·1 |
| PG[b] | 0·040 | 0·40 | 2·02 | | 19·1 |
| PI[b] | 0·043 | 0·42 | 2·25 | | 23·4 |
| PS[b] | 0·036 | 0·38 | 1·96 | | 19·6 |
| PC[b] | 0·039 | 0·34 | 1·72 | | 16·4 |

[a] Assume 3 moles hexose per mole of lipid.
[b] PA = phosphatidic acid, PE, PG, PI, PS, PC = phosphatidyl-ethanolamine, -glycerol, -inositol, -serine, -choline, respectively.

phosphatidic acid (PA) (5·5% of total PL). The small quantity of phosphatidic acid found was attributed to the enzymic hydrolysis of the phospholipids during the drying period (Kates, 1960*b*; Haverkate & Van Deenen, 1965; Yang *et al.*, 1967). The main fatty acids of phospholipids were palmitic, oleic and linoleic acids (Table 2). However, no trienoic acid was detected. Phospholipids were present in small amounts (0·20% of dried leaves).

## Non-polar lipids (NPL)

The components of this fraction were separated by TLC and identified with the aid of pure compounds. The compounds identified were sterols, steryl esters, fatty alcohols, free fatty acids, waxes, traces of triglycerides, triterpenic acids and components of the essential oil. The predominant component of the essential oil was carvacrol which exhibits pharmaceutical properties (anthelminthic). The essential oil used as a standard

**TABLE 2**
Fatty Acid Composition of *O. dictamnus* Lipids

| *Lipid* | *Fatty acids (relative area %)* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *14:0* | *16:0* | *16:1* | *18:0* | *18:1* | *18:2* | *18:3* |
| Non-polar lipids | 3·0 | 42·2 | 2·3 | 8·4 | 16·2 | 5·6 | 22·2 |
| Free fatty acids | 2·4 | 38·4 | 8·8 | 9·2 | 20·7 | 3·0 | 17·5 |
| Triglycerides | 5·9 | 51·9 | 3·6 | 4·3 | 22·7 | 10·9 | 0·8 |
| MDGD | 3·3 | 50·6 | 2·3 | 1·4 | 18·1 | 20·4 | 4·0 |
| CER | 2·2 | 40·7 | 2·4 | 7·7 | 29·1 | 17·7 | 1·7 |
| DGDG | 7·8 | 36·6 | 1·6 | 3·8 | 18·1 | 31·6 | 0·5 |
| SL | 0·5 | 48·0 | 2·8 | 5·2 | 25·1 | 19·1 | Trace |
| PE | 1·0 | 55·7 | 2·0 | 4·0 | 19·9 | 12·9 | 4·6 |
| PG | 2·0 | 48·0 | 7·7 | 4·2 | 15·2 | 17·2 | 5·6 |
| PI | 1·1 | 49·6 | 4·2 | 5·3 | 17·8 | 19·5 | 2·0 |
| PS | 4·4 | 44·3 | 2·8 | 4·3 | 19·8 | 16·4 | 3·0 |
| PC | 1·7 | 48·3 | 2·5 | 4·2 | 19·9 | 22·2 | 1·0 |

was obtained from dried leaves of *O. dictamnus* by steam distillation. Table 2 shows the composition of fatty acids of the non-polar lipids.

Triterpenic acid had the same $R_f$ as ursolic acid and gave violet colours upon spraying with the Brieskorn–Briner (Brieskorn *et al.*, 1954) reagent. Quantitative determination of triterpenic acid showed (Huang *et al.*, 1961; Christie, 1973) that they constituted 1·8% of the leaves.

## REFERENCES

Allen, C. F., Good, P., Davis, H. F., Chisum, P. & Fowler, S. S. D. (1963). Methodology for the separation of plant lipids and application to spinach leaf and chloroplastic lamellae. *J. Am. Oil Chem. Soc.*, **43**, 223–31.

Barlette, G. R. (1959). Phosphorus assay in column chromatography. *J. Biol. Chem.*, **234**, 466–8.

Beiss, U. (1964). Zur Papierchromatographischen Auftrennung von Pflanzenlipiden. *J. Chromatog.*, **13**, 104–10.

Brieskorn, C. H., Briner, M. & Leiner, U. (1954). Colorimetric determination of the total triterpenic acid content of Labiatae. *Arch. Pharm.*, **24**, 429–32.

Christie, W. (1973). *Lipid analysis*, Vol. 6, Pergamon Press, Oxford, p. 166.

Clayton, T. A., MacMurray, T. A. & Morrison, W. R. (1970). Identification of wheat flour lipids by thin layer chromatography. *J. Chromatog.*, **47**, 277–81.

Dittmer, J. D. & Lester, R. L. (1964). A simple specific spray for the detection of phospholipids on thin layer chromatograms. *J. Lipid Res.*, **5**(1), 126–7.

Fedelli, E. & Jacini, G. (1971). Lipid composition of vegetable oils. *Adv. Lipid Res.*, **9**, 335–82.

Fewster, M. E., Burns, B. J. & Mead, J. F. (1969). Quantitative densitometric thin layer chromatography of lipids using copper acetate reagent. *J. Chromatog.*, **43**, 120–6.

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the estimation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497–510.

Galliard, T. (1968). Aspects of lipid metabolism in higher plants. Identification and quantitative determination of the lipids in potato tubers. *Phytochemistry*, **7**, 1907–14.

Hansen, S. A. (1975). Thin layer chromatographic method for the identification of mono, di- and trisaccharides, *J. Chromatog.*, **107**, 224–6.

Haverkate, F. & Van Deenen, L. L. M. (1965). Isolation and chemical characterization of phosphatidyl glycerol from spinach leaves. *Biochim. Biophy. Acta*, **106**, 78–92.

Hitchock, C. & Nickols, B. W. (1971). *Plant biochemistry* (Sutcliff, G. F. and Halberg, P. (Eds)), Academic Press, London.

Huang, T. C., Chen, C. P., Welfer, V. & Raftery, A. (1961). A stable reagent for the Liebermann–Burchard reaction. Application to rapid serum cholesterol determination. *Anal. Chem.*, **33**(10), 1405–7.

Jacin, H. & Mishkin, A. R. (1965). Separation of carbohydrates in borate impregnated silica gel G plates. *J. Chromatog.*, **18**, 170–3.

Kates, M. (1960*a*). *Lipid metabolism* (Bloch, J. (Ed.)), J. Wiley, New York, pp. 161–237.

Kates, M. (1960*b*). Chromatographic and radioisotopic investigations of the lipid components of runner bean leaves. *Biochim. Biophy. Acta*, **41**, 315–28.

Kates, M. (1972). *Techniques in lipidology*, North-Holland Publishing Co., Amsterdam.

Kavvadas, D. S. (1956). *Botanical and phytological vocabulary*, Athens, p. 686.

Leese, B. M. & Leech, R. M. (1976). Sequential changes in the lipids of developing proplastids isolated from green maize leaves. *Plant Physiol.*, **57**, 789–94.

Lepage, M. (1964). Isolation and characterization of an esterified form of steryl glucoside. *J. Lipid Res.*, **5**(4), 587–92.

Mangold, H. K. (1969). *Thin layer chromatography* (Stahl, E. (Ed.)), Springer Verlag, New York, pp. 263–421.

Marinetti, G. (1964). *New biochemical separations*, Van Nostrand, New York, p. 339.

Morrison, W. R. & Smith, L. M. (1964). Preparation of fatty acid methylesters and dimethylacetals from lipids with boron fluoride–methanol. *J. Lipid Res.*, **5**, 600–8.

Nickols, B. W. (1964). *New biochemical separations*, Van Nostrand, New York, pp. 34–41.

*Official Journal of the European Communities* (O.J.E.C.), 24-5-1977, 128/20.
Pohl, P., Glasl, H. & Wagner, H. (1970). Zur Analytik Pflanzlicher Glyko-und Phospholipoide und ihrer Fettsäuren. *J. Chromatog.*, **49**, 488–92.
Roughan, P. G. & Batt, R. D. (1968). Quantitative analysis of sulpholipid (sulphoquinovosyl diglyceride) and galactolipids (monogalactosyl and digalactosyl diglyceride) in plant tissue, *Anal. Biochem.*, **22**, 74–8.
Roughan, P. G. & Batt, R. D. (1969). The glycerolipid composition of leaves. *Phytochemistry*, **8**, 363–9.
Rouser, G., Kritchevsky, G. & Yamamoto, A. (1967*a*). *Lipid chromatographic analysis*, Dekker Inc., New York, pp. 99–162.
Rouser, G., Kritchevsky, G. & Yamamoto, A. (1967*b*). *Lipid chromatographic analysis*, Vol. 3 (Marinetti, G. (Ed.)), Dekker Inc., New York, pp. 713–89.
Rouser, G., Kritchevsky, G., Yamamoto, A., Simon, G., Galli, G. & Bauman, A. J. (1969). *Methods in enzymology*, **14**, 272–317.
Sapeica, N. (1969). *Food pharmacology*, Charles C. Tomas, Springfield.
Sastry, P. S. & Kates, M. (1964). Lipid component of leaves. V. Galactolipids, cerebrosides and lecithin of runner-bean leaves. *Biochemistry*, **3**(9), 1271–80.
Smith, C. R. & Wolff, I. A. (1966). Glycolipids of *Briza spicata* seed. *Lipids*, **1**(2), 123–7.
Svennerholm, L. (1956). The quantitative estimation of cerebrosides in nervous tissue. *J. Neurochem.*, **1**, 42–53.
Takeda, K., Hara, S., Wada, A. & Matsumoto, N. (1963). *J. Chromatog.*, **11**, 562–64.
Theodossiou, Ph. P. (1972). Contribution and l'etude des substances polyphenoliques de l'*Origanum dictamnus* L. *Archives de Pharmacie de la Societe Pharmaceutique Hellenique*, **1–3**.
Vitielo, F. & Zanetta, J. P. (1978). Thin layer chromatography of phospholipids. *J. Chromatog.*, **166**, 637–40.
Wagner, H., Horhammer, L. & Wolff, P. (1961). Dünnschicht chromatographie von Phosphatiden und Glykolipiden. *Biochem. Z.*, **334**, 175–84.
Wintermans, J. F. G. M. (1963). Phosphatides and glycolipids of photosynthetic apparatus. *Colog. Intern. Centre Natl. Rech. Sci.*, **119**, 381–5.
Yang, S. F., Freer, S. & Benson, A. A. (1967). Transphosphatidylation by phospholipase D. *J. Biol. Chem.*, **242**(3), 477–84.