ELSEVIER

Food Chemistry 76 (2002) 469–473

Food Chemistry

www.elsevier.com/locate/foodchem

Occurrence of dicarboxylic (dioic) acids in some Mediterranean nuts

Valery M. Dembitsky[a,*], Paulina Goldshlag[b], Morris Srebnik[a,c]

[a]Department of Medicinal Chemistry and Natural Products, School of Pharmacy, PO Box 12065, The Hebrew University of Jerusalem, Jerusalem, 91120, Israel

[b]Plant Protection and Inspection Service, Ministry of Agriculture, PO Box 78, Beth Dagan 50250, Israel

[c]David R. Bloom Center for Pharmaceutical Research, Hebrew University of Jerusalem, Jerusalem, Israel

Received 22 May 2001; received in revised form 30 July 2001; accepted 30 July 2001

Abstract

A GC–MS method was developed for the determination of 26 minor dicarboxylic (dioic) acids (from 1.87 to 7.79%) in seven species of Mediterranean nuts growing in Israel. The occurrence of hydroxy, methoxy, branched, and unsaturated dioic acids in nuts has been studied. Together with known acids ($C_{3:0}$–$C_{22:0}$), unusual dicarboxylic acids were identified by gas chromatography/mass spectrometry. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Mediterranean nuts; Hydroxy; Methoxy; Branched; Unsaturated dioic acids; GC–MS

1. Introduction

Nut and seed oils of different plants consist predominantly of saturated or unsaturated (olefinic) straight-chain C_{16}–C_{18} fatty acids with a terminal carboxyl group, which are esters of fatty acids with glycerol (Smith, 1971). Many minor unusual fatty acids have been identified in seed oils (Badami & Patil, 1982; Spitzer, 1999). At present, more than 1000 fatty acids have been isolated and their structure elucidated by GC–MS and/or NMR methods (Gunstone, 1999).

Growing interest in fatty acids with unusual structural features in plant oil is of interest in the chemical, pharmaceutical, and especially cosmetic industry (Derksen, Muuse, & Cuperus, 1994; Gunstone, 1999; Shukla & Kragballe, 1998).

Some dicarboxylic (dioic) acids have potential as antiproliferatives and, as general anti-tumor agents, for primary invasive malignant melanoma (Breathnach, 1999). Aliphatic dicarboxylic acids surprisingly afford potent cytotoxicity, anti-neoplastic activity (Hall et al., 1999), and served as lipidoic markers for identification of some human diseases (Ma, Baraona, Gooozner, & Lieber, 1999).

In the present investigation, we have attempted to characterize the dioic acid composition of seven Mediterranean nuts which grow in Israel.

* Corresponding author. Fax: +972-2-6450-276.
E-mail address: dvalery@cc.huji.ac.il (V.M. Dembitsky).

2. Materials and methods

2.1. Samples

All nuts were purchased in a local market in Jerusalem from February to August 2000.

2.2. Analytical procedure

The nuts were shelled, and then the clear kernels were separately homogenized in a high-speed unit, and the lipids extracted with a mixture of chloroform–methanol (2:1, v/v), according to an established procedure (Christie, 1989), followed by addition of a saturated solution of sodium chloride in water. The mixture was vigorously shaken for 30 s. After phase separation, the lower layer was removed, dried with sodium sulfate, and filtered. The oil residue was dissolved in CH_2Cl_2 and stored at −20 °C prior to GC–MS analysis.

GC–MS analysis was performed with a Hewlett Packard 6890 series II chromatograph linked to a HP 5973 mass detector, equipped with HP automatic injector and a 30 m long, 0.25 mm ID, 0.25 μm film thickness HP-5MS capillary column. The ionization energy was 70 eV. The carrier gas was He (flow 1.0 ml/min). The temperature of the injection block was 250 °C. The GC oven temperature was programmed as follows: initial temperature 60 °C (2 min), followed by a temperature increase of 3 °C/min up to 260 °C (10 min) and second rate of 10 °C/min to the final temperature of 290 °C (10

0308-8146/02/$ - see front matter
PII: S0308-8146(01)00308-9

min). Identification of the compounds was established using the *Wiley 7th Edition Library*.

3. Results and discussion

GC–MS analysis of the total dioic and fatty acid compositions of seven nut oils resulted in the identification of 50 different acids. The structure of all dioic acids was established using GC–MS, using the dimethyl ester derivatives. A series of 26 dioic (dicarboxylic) acids was also identified in the nut oils, and these accounted for 1.87 to 7.79% of the total fatty acid composition (Table 1). An unusual finding was the identification of dioic acids, such as (*E*)-2-butene-1,4-dioic, 2-hydroxybutane-1,4-dioic, 2-methylbutane-1,4-dioic acid, methylene-butane-1,4-dioic, and other dioic acids. None of these minor dioic acids had been found previously in nuts.

Dimethyl esters of dioic acids have mass spectra that are different in appearance and more complex than their monocarboxylic analogues. The 70 eV mass spectra of all dioic acids were characterized by a molecular ion $[M-31]^+$ peak, which is due to the loss of the methoxyl group (H_3CO) (McLafferty & Turecek, 1993; Murphy, 1993). In the mass spectra of long acid (from C_{13} to C_{22}) dimethyl esters, the base peak ion is m/z 98 (Rezanka, 1998). The molecular ion is of low abundance and is not seen in mass spectra of all dioic acids. The m/z values of separated ions including their relative abundance are listed in Table 2. Fig. 1 shows the GC–MS chromatogram and Fig. 2 presents representa-tive structures of twenty six identified dioic acids.

Throughout history, nuts have been a staple food, providing energy, essential fatty acids, protein, vitamins, and minerals. At present, nuts are classified as part of the USDA Food Guide Pyramid's Meat/Meat Alternate Group (Dreher, Maher, & Kearney, 1996). Nuts are also being studied for their potential health benefits. Research suggests that there may be a connection between frequent nut consumption and a reduced incidence of coronary heart disease (Dreher et al., 1996; Fraser, 2000).

Natural dicarboxylic acids are major components of plant polymeric compounds such as cutin and suberin (Holloway, 1984; Walton, 1990).

Low molecular weight dicarboxylic acids were found in rhizosphere soil of durum wheat (Szmigielska, Van Rees, Cieslinski, & Huang, 1996), in vegetables (Siddiqui, 1989), and long dioic acids in some lichenized fungi (Dembitsky, 1992; Sassaki, Cruz, Gorin, & Iacomini, 2001). More than 26 dioic acids (C_{10}–C_{35}) were identified in spores of an ancient fern group, *Equisetum* (Rezanka, 1998). Dioic acids are also found in birch and in the inner and outer bark of spruce and pine (Ekman & Reunanen, 1983). However, the greatest diversity of

Table 1
Distribution of dicarboxylic (dioic) acids in some Mediterranean nuts (wt.%)

Fatty acids	Almond	Hazelnut	Peanut	Pecan	Pinenut	Pistachio	Walnut
1. Propane-1,3-dioic	0.14	1.02	1.49	0.19	0.20	0.17	0.34
2. Butane-1,4-dioic	0.32	0.74	1.31	0.08	0.35	0.59	0.90
3. (*E*)-2-Butene-1,4-dioic		0.36	0.44			0.12	
4. (*Z*)-2-Butene-1,4-dioic					0.09		0.22
5. 2-Methylene-Butane, 1,4-dioic				0.06		0.37	
6. (*R*)-Methyl-Butane-1,4-dioic		0.09	0.29				0.05
7. 2(3)-Hydroxy-Butane-1,4-dioic	0.53	0.93	1.29	0.32	2.14	3.45	1.51
8. 2,3-Dihydroxy-Butane-1,4-dioic			0.35		0.42		0.04
9. 2-Methoxy-2,Butene-1,4-dioic			0.13				0.20
10. Pentane-1,5-dioic	0.13	0.09	0.45		0.22		
11. Hexane-1,6-dioic		0.07	0.06	0.07		0.04	0.02
12. Heptane-1,7-dioic	0.11	0.14	0.09				0.07
13. Octane-1,8-dioic	0.07	0.22	0.25		0.14		0.15
14. Nonane-1,9-dioic	0.22	1.45	1.06	0.61	0.78	1.36	1.18
15. Decane-1,10-dioic		0.09	0.05			0.04	
16. Undecane-1,11-dioic	1.98				0.51		
17. Dodecane-1,12-dioic	0.24			0.19		0.09	
18. Tridecane-1,13-dioic		0.45	0.21				
19. Tetradecane-1,14-dioic			0.27				0.14
20. Pentadecane-1,15-dioic		0.14			0.18		
21. Hexanedecane-1, 16-dioic					0.24		
22. Heptadecane-1,17-dioic	0.31		0.04			0.27	
23. Octadecane-1,18-dioic		0.12		0.09	0.03		0.18
24. Nonadecane-1,19-dioic				0.13			
25. Icosane-1,20-dioic		0.08	0.01	0.05			0.03
26. Docosane-1,22-dioic		0.06		0.08			0.11
Total dioic acids	4.07	6.05	7.79	1.87	5.35	6.50	5.14

Table 2
Content, structure and abundance of major ions from dimethyl esters of dioic acids[a]

No.[a]	M,[b] (m/z, relative abundance)	$[M-31]^+$	$[M-32]^+$	$[M-64]^+$	$[M-73]^+$	$[M-92]^+$	$[M-105]^+$	$[M-123]^+$	Other major peaks
1.	132(1)[c]	101(100)							101(100) 59(90)
2.	146(0.5)	115(100)	114(35)						115(100), 55(35)
3.	144(0.3)	113(100)	112(0.2)						85(50)
4.	144(0.8)	113(100)							85(20), 59(29)
5.	158(15)	127(78)	126(54)						113(24), 99(52), 59(100)
6.	160(0.1)	129(55)	128(33)						101(24), 88 (20), 59 (100)
7.	162(0.5)	131(1)							103(100), 71(69), 43(65)
8.	178(0.1)	147(0.1)							119(39), 90 (100), 33(56)
9.	176(0.2)	145(1)	144(4)						117(50), 75 (100)
10.	160(0.3)	129(50)	128(20)						59(100) 100(62) 129 (60)
11.	174(0.1)	143(30)	142(10)						59(100) 55(70) 114(60)
12.	188(0.1)	157(25)	156(7)	124(18)	115(68)	96(10)	83(41)		59(100), 74(48) 55(60)
13.	202(0.6)	171(71)	170(6)	138(92)	129(100)	110(21)	97(42)		55(45), 59 (31), 74(42)
14.	216(0.2)	185(32)	184(6)	152(56)	143(29)	124(19)	111(44)	93(2)	55(100), 74(90), 83(60)
15.	230(0.2)	199(38)	198(3)	166(23)	157(38)	138(26)	125(52)	107(5)	55(100), 59(48), 74(89)
16.	244(0.3)	213(28)	212(1)	180(6)	171(27)	152(22)	139(46)	121(11)	55(100), 74(100), 98(74)
17.	258(0.2)	227(23)	226(1)	194(8)	185(18)	166(6)	153(29)	135(16)	55(100), 74(84), 98(75)
18.	272(0.1)	241(8)	240(1)	208(4)	199(16)	180(2)	167(4)	149(7)	98(100), 74(91), 55(74)
19.	286(0.6)	255(32)	254(2)	222(8)	213(34)	194(6)	181(19)	163(13)	98(100), 74(76), 55(58)
20.	300(0)	269(49)	268(3)	236(25)	227(86)	208(9)	195(100)	177(94)	98(100), 74(68), 55(47)
21.	314(8)	283(26)	282(5)	250(9)	241(30)	222(2)	209(22)	191(15)	98(100)
22.	328(0)	297(69)	296(2)	264(12)	273(79)	236(3)	223(7)	205(77)	123(100), 122(66)
23.	342(1)	311(24)	310(6)	278(5)	269(22)	250(4)	237(10)	219(4)	98(100), 112(38)
24.	356(0)	325(28)	324(6)	292(6)	283(20)	264(4)	251(11)	233(5)	98(100), 112(36)
25.	370(0)	339(6)	228(1)	306(8)	297(4)	278(2)	265(7)	247(3)	98(100), 112(40)
26.	398(3)	367(27)	366(14)	334(9)	325(21)	306(6)	293(12)	275(4)	98(100), 112(34)

[a] No., Number and name of dioic acids are in Table 1.
[b] M, for dimethyl ester of appropriate in dioic acids.
[c] % of base peak.

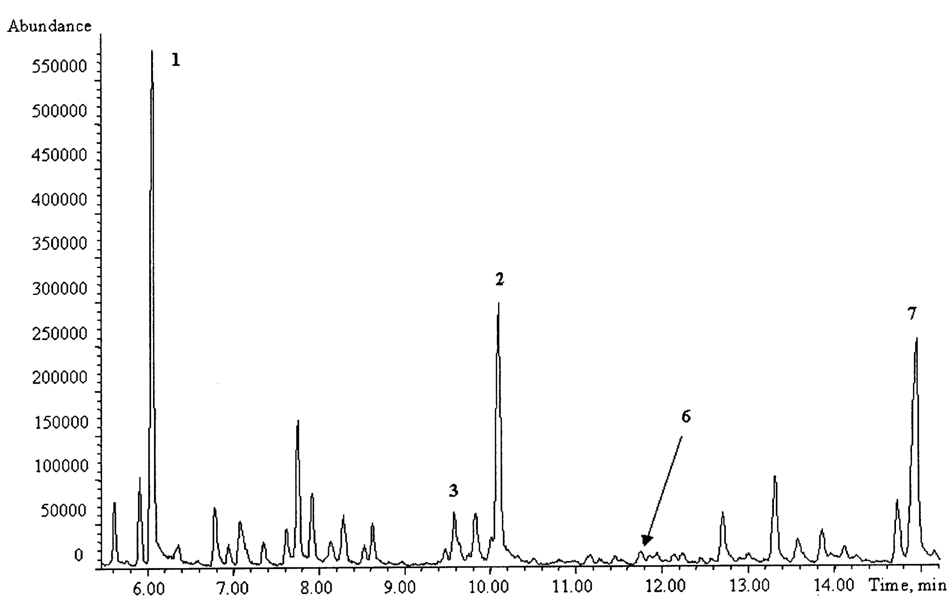

Fig. 1. Part of gas chromatographic (full run is 121 min) separation of some low molecular dimethyl esters of dioic acids identified from hazelnut oil. Compounds associated with the numbered peaks are given in Table 1.

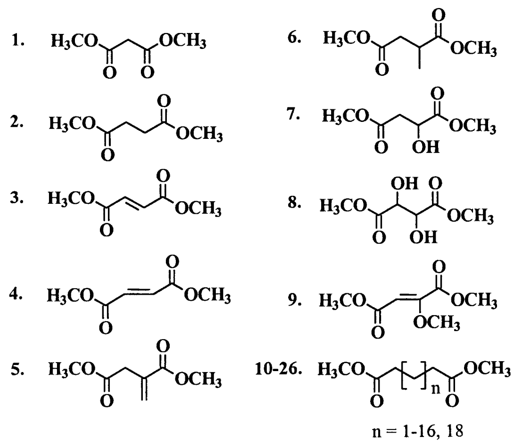

Fig. 2. Dicarboxylic (dioic) acids identified from Mediterranean nuts. For acid names refer to Table 1.

dicarboxylic acids was found among hyperthermophilic micro-organisms and in bacteria (Carballeira, Reyes, Sostre, Huang, Verhagen, & Adams, 1997). Recently, we have studied the composition of fatty acids for four strains of cyanobacteria belonging to the genus *Aphanizomenon* and found some unusual dicarboxylic acids (Dembitsky, Shkrob, & Go, 2001).

Dicarboxylic acids are a suitable and easily available substrate for preparation of organic acids for the pharmaceutical and food industries. Chemical synthesis and/or microbial transformations of these acids were recently reviewed by Mikova, Rosenberg, and Kristofikova (2001). These acids are of major interest for medical specialists.

Thus, treatment, by derivatives of hexade-canedioic acid, of an animal model (rats) for obesity/insulin-resistant/hyperlipidemic syndrome not only markedly improved lipid metabolism, but also inhibited the development of advanced cardiovascular disease (Russell, Dolphin, Hameed, Stewart, Koesdlag, Rosekahn, & Bartana, 1991; Russell, Amy, Graham, Dolphin, Wood, & Bartana, 1995).

High diversity of butanedioic acids (eight species) in nuts could be explained by an important physiological role of these acids in the photosynthetic apparatus in chloroplasts (Ivanishchev, 1997; Pfundel, Nagel, & Meister, 1996).

The results of these studies have shown, for the first time, the presence of 26 dioic acids in Mediterranean nuts.

References

Badami, R. C., & Patil, K. B. (1982). Structure and occurrence of unusual fatty acids in minor seed oils. *Progress in Lipid Research*, *19*, 119–153.

Breathnach, A. S. (1999). Azelaic acid: potential as a general antitumoural agent. *Medical Hypothesis*, *52*, 221–226.

Carballeira, N. M., Reyes, M., Sostre, A., Huang, H., Verhagen, M. F. J. M., & Adams, M. W. W. (1997). Unusual fatty acid compositions of the hyperthermophilic archaeon *Pyrococcus furiosus* and the bacterium *Thermotoga maritima*. *Journal of Bacteriology*, *179*, 2766–2768.

Christie, W. W. (1989). *Gas chromatography and lipids. A practical guide*. Ayr: The Oil Press.

Dembitsky, V. M. (1992). Lipids of lichens. *Progress in Lipid Research*, *31*, 373–397.

Dembitsky, V. M., Shkrob, I., & Go, J. V. (2001). Dicarboxylic and fatty acid compositions of the genus *Aphanizomenon*. *Biochemistry (Moscow)(Translation of Biokhimiya (Moscow)), Eng. Ed*, *66*, 72–76.

Derksen, J. T. P., Muuse, B. G., & Cuperus, F. P. (1994). Processing of novel oil crops and seed oils. In D. J. Murphy (Ed.), *Designer oil crops* (pp. 253–281.

Dreher, M. L., Maher, C. V., & Kearney, P. (1996). The traditional and emerging role of nuts in healthful diets. *Nutrition Reviews*, *54*, 241–245.

Ekman, R., & Reunanen, M. (1983). Composition of aliphatic monomers from the outer bark suberin of Norway spruce and scots pine. *Finn Chemical Letters*, *10*, 166–168.

Fraser, G. E. (2000). Nut consumption, lipids, and risk of a coronary event. *Asia Pacific Journal of Clinical Nutrition*, *9*, S28–S32.

Gunstone, D. F. (1999). *Fatty acid and lipid chemistry*. USA: Garfield, Aspen Publ., Inc.

Hall, I. H., Izydore, R. A., Warren, A. E., & Barnes, C. R. (1999). Cytotoxicity and mode of action of aliphatic dicarboxylic acids in L1210 lymphocytic leukemia cells. *Anticancer Research*, *19*, 205–211.

Holloway, P. J. (1984). Cutins and suberins, the polymeric plant lipids. In H. K. Mangold, G. Zweig, & J. Sherma (Eds.), *CRC handbook of chromatography lipids (Vol. 1)* (pp. 321–345.

Ivanishchev, V. V. (1997). Biological role of oxaloacetate metabolism in chloroplasts of C-3 plants. *Russian Journal of Plant Physiology*, *44*, 401–408.

Ma, X., Baraona, E., Goozner, B. G., & Lieber, C. S. (1999). Gender differences in medium-chain dicarboxylic aciduria in alcoholic men and women. *American Journal of Medicine*, *106*, 70–75.

McLafferty, E. W., & Turecek, F. (1993). *Interpretation of mass spectra* (4th ed.). Sausalito, California: University Science Books.

Mikova, H., Rosenberg, M., & Kristofikova, L. (2001). Production of dicarboxylic acids important for technology. *Chemicke Listy*, *95*, 28–33.

Murphy, R. C. (1993). *Mass spectrometry of lipids. Handbook of Lipid Research 7*. NY: Plenum Press.

Pfundel, E., Nagel, E., & Meister, A. (1996). Analyzing the light energy distribution in the photosynthetic apparatus of C-4 plants using highly purified mesophyll and bundle-sheath thylakoids. *Plant Physiology*, *112*, 1055–1070.

Rezanka, T. (1998). Branched and very long-chain dicarboxylic acids from *Equisetum* species. *Phytochemistry*, *47*, 1539–1543.

Russell, J. C., Amy, R. M., Graham, S. E., Dolphin, P. J., Wood, G. O., & Bartana, J. (1995). Inhibition of atherosclerosis and myocardial lesions in the JCR-LA-CP rat by β,β′-tetramethylhexadecanedioic acid (Medica-16). *Arteriosclerosis Thrombosis and Vascular Biology*, *15*, 918–923.

Russell, J. C., Dolphin, P. J., Hameed, M., Stewart, B., Koeslag, D. G., Rosekahn, G., & Bartana, J. (1991). Hypolipidemic effect of β,β′-tetramethyl hexadecanedioic acid (Medica-16) in hyperlipidemic JCR-LA-corpulent rats. *Arteriosclerosis and Thrombosis*, *11*, 602–609.

Sassaki, G. L., Cruz, L. M., Gorin, P. A. J., & Iacomini, M. (2001). Fatty acid composition of lipids present in selected lichenized fungi: a chemotyping study. *Lipids*, *36*, 167–174.

Shukla, V. K. S., & Kragballe, K. (1998). Exotic butters as cosmic lipids. *Inform*, *9*, 512–516.

Siddiqui, I. R. (1989). Studies on vegetables- fiber content and chemical-composition of ethanol-insoluble and ethanol-soluble residues. *Journal of Agriculture and Food Chemistry*, *37*, 647–650.

Smith, C. R. J. (1971). Occurrence of unusual fatty acids in plants. *The Chemistry of Fats and Other Lipids*, *11*, 137–177.

Spitzer, V. (1999). Screening analysis of unknown seed oils. *Fett/Lipid*, *101*, 2–19.

Szmigielska, A. M., Van Rees, K. C. J., Cieslinski, G., & Huang, P. M. (1996). Low molecular weight dicarboxylic acids in rhizosphere soil of durum wheat. *Journal of Agricultural and Food Chemistry*, *44*, 1036–1040.

Walton, T. J. (1990). Waxes, cutin and suberin. In *Methods in plant biochemistry* (Vol. 4, pp. 105–158). San Diego: Academic Press.