4. Eisner, A., T. Perlstein and W.C. Ault, Ibid. 39:290 (1962).
5. Eisner, A., T. Perlstein and W.C. Ault, Ibid. 40:594 (1963).
6. Smith, F.D., H.E. Kenney and A.J. Stirton, J. Org. Chem. 30:885 (1965).
7. Smith, F.D., A.J. Stirton and M.V. Nuñez-Ponzoa, JAOCS 43:501 (1966).
8. Smith, F.D., A.J. Stritton and M.V. Nuñez-Ponzoa, Ibid. 44: 413 (1967).
9. Smith, F.D., A.J. Stirton and C.J. Dooley, Ibid. 45:747 (1968).
10. Smith, F.D., and A.J. Stirton, Ibid. 48:160 (1971).
11. Showell, J.S., D. Swern and W.R. Noble, J. Org. Chem. 33: 2697 (1968).
12. Solomon, H.L., W.D. Hubbard, A.R. Prosser and A.J. Sheppard, JAOCS 51:424 (1979).

[Received August 19, 1983]

The Fatty Acid Composition of Gymnospermae Seed and Leaf Oils

J.R. VICKERY, F.B. WHITFIELD, G.L. FORD and **B.H. KENNETT**, CSIRO Division of Food Research, PO Box 52, North Ryde, NSW 2113, Australia.

ABSTRACT

Of 12 Gymnospermae seed and leaf oils, only 2 contained cyclopropene fatty acids. All-*cis* 5, 11, 14, 17-eicosatetraenoic acid occurred in concentrations up to 11.9% in 6 seed oils, and up to 61% in 2 leaf oils. The structure of this acid, as its methyl ester, was established by the combination of physical (UV, IR, ^{1}H- and ^{13}C-NMR and mass spectra) and chemical techniques. Arachidonic acid also occurred in 2 seed oils.

INTRODUCTION

Berry (1) first reported the occurrence of cyclopropene fatty acids (CPFA) in Gymnospermae species, finding 40–50% in the seed and leaf oils of *Gnetum gnemon;* a tree widely cultivated in Southeast Asia. Takagi and Itabashi (2) reported significant amounts of non-methylene-interrupted polyenoic acids in various species in the Cupressaceae, Pinaceae, Podocarpaceae and Taxodiaceae families, including up to 8.5% of all-*cis* 5, 11, 14, 17-eicosatetraenoic acid in the seed oils of 3 species in each of the Cupressaceae and Taxodiaceae families.

All-*cis* 5, 8, 11, 14- and all-*cis* 5, 11, 14, 17-eicosatetraenoic acids were found by Schlenk and Gellerman (3) in mosses and in the leaves and seeds of *Ginkgo biloba.* Kleiman et al. (4) identified the all-*cis* 5, 11, 14, 17-acid in the seed oil of *Ephedra campylopoda,* while Jamieson and Reid (5) found up to 7.7% of this acid in the leaf acids of 33 conifer species.

The fatty acids of the seed oils of 9 Australian Gymnospermae species and 3 leaf oils have been assayed for the presence of CPFA and eicosatetraenoic acids.

EXPERIMENTAL PROCEDURES

Material

Seeds and leaves were collected in New South Wales, Queensland and Tasmania.

General Procedures

The methods used for the extraction of the oils, the preparation of methyl esters, the Halphen color test, the determination of CPFA and the gas chromatography procedures were similar to those described in two previous papers (6, 7).

Separation and Identification of Methyl Eicosatetraenoate

The methyl ester was separated in 80–90% purity by fractionation of the ester mixtures on a column (30 cm X 1.5 cm) of Adsorbosil CABN containing 25% silver nitrate (Applied Science Laboratories, State College, PA) using a series of eluting solvents—toluene-hexane (50:50, v/v), toluene-hexane (75:25, v/v), toluene (100%) and ether-hexane (25:75, v/v). Most of the ester appeared in the 2 later eluents. The purity of these fractions was raised to 95–98% by fractionation on TLC plates, 0.5 mm thick, prepared from Silica gel PF_{254} (E. Merck AG, Darmstadt, W. Germany) containing 16% silver nitrate, using ether-hexane (12:88, v/v) at 20 C as the developing solvent. Alternately, toluene-hexane (90:10, v/v) at −20 C was used. The tetraenoate occurred in the narrow band remaining at the origin. Samples of the ester for spectroscopic examination were further purified by gas liquid chromatography (GLC) using a stainless steel capillary column (150 m long and 0.75 mm i.d.), the walls coated with Silicone OV-101. The ultraviolet (UV) spectrum of the ester (in hexane) was recorded on a Gilford 2600 spectrophotometer, the infrared (IR) spectrum (as a thin film) on a Perkin Elmer 521 spectrometer, and the ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra (as C_6D_6 solutions) on a Bruker CXP100 spectrometer. The low-resolution and high-resolution mass spectra were recorded using a Varian MAT-311A mass spectrometer. The products obtained from the hydrazine reduction (8) of the tetraenoate were isolated by argentation TLC at −20 C (9) and were identified by GLC and mass spectrometry. Products obtained from the oxidative cleavage (10) of the monoenoates derived from the partial reduction of the tetraenoate were identified by GLC using a glass column (4 m long, 2 mm i.d.) packed with 10% Silar 10C on Gas-Chrom Q. The dimethyl dicarboxylates were identified by comparison of their GLC retention times with those of a standard ester mixture.

RESULTS AND DISCUSSION

The results of the assay of the methyl esters and the average mass and oil contents of the air-dried seeds without testa are given in Table I.

Oil Contents

The oil contents of the seeds ranged from high (13.5%) in *Athrotaxis selaginoides* to low values (0.6–1%) in *Macrozamia communis* and *Podocarpus elatus.*

Cyclopropene Fatty Acids

Malvalic and sterculic acids occurred in moderate amounts in only 2 species — *Macrozamia communis* and *Callitris rhomboidea.* These results, together with those previously reported by Vickery (6), indicate that the occurrence of CPFA is erratic in most plant families except Malvaceae, Sterculiaceae and Bombacaceae.

TABLE I

Halphen Test, Oil Contents and Methyl Esters for 12 Gymnosperm Seed and Leaf Oils

Family	Cupressaceae					Podocarpaceae		Taxodiaceae				Zamiaceae
Species	*Callitris columellaris* F. Muell.	*Callitris endlicheri* (Parl.)F.M.Bail.	*Callitris oblonga* A.etL.C.Rich.	*Callitris rhomboidea* R.Br.exA.et L.C.Rich.		*Podocarpus elatus* R.Br.ex Endl.	*Podocarpus lawrencei* Hook f.	*Athrotaxis cupressoides* Don.		*Athrotaxis selaginoides* Don.		*Macrozamia communis* L. Johnson
	seed	seed	seed	seed	leaf	seed	seed	seed	leaf	seed	leaf	seed
Halphen test	–	–	–	+	–	–	–	–	–	–	–	+
Oil content (%)	3.2	2.9	7.6	4.7	4.6	1.0	8.0	7.4	4.6	13.5	15.6	0.6
No. seeds/g	100	125	250	5	–	3	50	830	–	1050	–	0.2
Methyl esters, mass (%)												
Satd. 10:0 + 12:0	–	–	–	–	0.8	–	–	–	6.4	–	0.6	–
14:0	0.2	–	–	0.2	6.9	0.2	7.4	0.1	7.8	0.4	0.4	0.5
15:0	–	–	–	–	–	–	–	–	1.5	–	–	–
16:0	8.1	6.2	4.6	5.6	10.6	17.4	9.0	6.5	12.5	6.8	5.2	17.2
17:0	–	–	–	–	–	1.7	–	–	1.5	–	–	–
18:0	3.8	3.2	2.5	2.4	3.4	4.9	3.2	2.4	3.2	2.7	0.2	2.3
19:0	–	0.9	–	0.5	–	–	–	–	–	–	–	1.5
20:0	0.5	0.9	1.8	0.4	–	1.7	–	0.9	–	–	–	1.3
22:0	0.4	–	–	0.8	–	–	–	–	–	–	–	–
Unsatd. 14:1	–	–	–	0.1	1.3	–	–	–	–	–	–	–
15:3	–	–	–	–	–	–	–	–	–	–	37.5	–
16:1	0.4	0.5	0.8	0.5	2.5	1.4	–	1.9	0.8	0.2	1.1	2.1
18:1	12.7	15.7	10.4	9.9	11.1	38.7	34.7	12.7	2.3	16.0	6.2	29.3
18:2	20.8	25.8	21.6	29.9	29.3	24.3	24.9	31.5	1.4	32.2	13.9	28.0
18:3	36.1	27.6	34.0	25.4	–	3.7	8.4	32.7	0.5	34.3	3.7	3.2
20:1	0.4	0.5	2.8	0.6	8.1	1.1	–	0.9	0.6	0.7	–	0.4
20:2	1.7	1.2	1.5	1.9	–	–	2.1	3.1	–	1.4	0.4	0.2
20:3	2.5	4.6	4.0	0.3	14.4	1.6	5.6	–	0.4	0.5	0.5	1.3
20:4[a]	10.2	9.3	11.9	–	–	–	4.7	4.8	61.1	4.8	24.6	–
20:4[b]	–	–	–	–	2.1	1.2	–	0.7	–	–	–	–
20:5	0.2	–	–	–	2.0	–	–	–	–	–	–	–
22:1	1.3	2.0	1.2	0.5	7.5	–	–	0.1	–	–	0.9	–
22:2	–	–	–	–	–	–	–	0.2	–	–	0.8	–
22:3	–	–	–	–	–	–	–	0.7	–	–	2.4	–
22:4	–	–	–	–	–	–	–	–	–	–	1.1	–
malvalic	–	–	–	6.8	–	–	–	–	–	–	–	10.7
sterculic	–	–	–	12.3	–	–	–	–	–	–	–	0.4
DHM[c]	0.1	0.2	0.6	0.2	–	–	–	–	–	–	–	0.2
DHS[d]	0.6	1.4	2.1	1.6	–	0.7	–	0.7	–	–	0.5	1.3

[a]All-*cis* 5, 11, 14, 17

[b]All-*cis* 5, 8, 11, 14

[c]Dihydromalvalic

[d]Dihydrosterculic

Eicosatetraenoic Acids

Two 20:4 acids were detected, the all-*cis* 5,8,11,14- identified, as its methyl ester, by comparison with an authentic sample of methyl arachidonate and the all-*cis* 5,11,14,17-. The structure of the latter acid, as its methyl ester, was established by the combination of physical and chemical techniques. The GLC-pure methyl ester gave a low-resolution mass spectrum with prominent and diagnostic ions as follows: M/Z 318 M^+(2), 108(45), 95(52), 93(47), 81(49), 80(48), 79(100), 67(83), 55(54), 41(78). The high-resolution mass spectral examination gave a molecular weight of 318.2556, which was consistent only with the molecular formula $C_{21}H_{34}O_2$ (calc. 318.2559). The UV and IR spectra showed the absence of conjugated unsaturation and *trans*-olefinic groups. The ^{1}H-NMR spectrum had a complex multiplet at δ 5.41 (8H) assigned to 8 olefinic protons (11) and the ^{13}C-NMR spectrum had 8 signals between δ 127.03 and 132.09, which appeared as doublets in the off-resonance decoupled mode, assigned to 8 alkyl-substituted olefinic carbon atoms (12). These NMR spectra were essentially identical to those of the published spectra of methyl all-*cis* 5,11,14,17-eicosatetraenoate (2). The position of the double bonds in the tetraenoate were confirmed as follows. Partial reduction with hydrazine gave a mixture of 4 isomeric methyl 20:1 esters. Oxidative fragmentation of the individual monoenoates followed by methylation of the oxidation products gave 4 dimethyl dicarboxylates containing, respectively, 5, 11, 14 and 17 carbon atoms. These fragmentation products showed the 20:4 methyl ester had double bonds at carbon atoms 5, 11, 14 and 17.

Up to 11.9% of the all-*cis* 5, 11, 14, 17- acid occurred in the seed oils of 6 species in the present study (Table I), but much higher amounts, up to 61%, occurred in the leaf oils of the two *Athrotaxis* species.

Only 3 occurrences of the all-*cis* 5, 8, 11, 14-acid were found in *Callitris rhomboidea* leaf oil and in *Podocarpus elatus* and *Athrotaxis cupressoides* seed oils, where both 20:4 acids occurred. Analysis of the latter oil using a 40 m glass capillary column coated with Silar 10-C gave E.C.L. values of 21.93 and 22.48 for the methyl 5,8,11,14- and the 5,11,14,17-eicosatetraenoates, respectively.

Other Acids

Dihydrosterculic acid in amounts up to 2.1% was present in most oils. Dihydromalvalic acid occurred in small amounts in 4 seed oils. The concentrations of linoleic plus linolenic acids exceeded 53% in the seed oils of four *Callitris* and two *Athrotaxis* species, being 66.5% in *Athrotaxis selaginoides*. In the two *Podocarpus* species, oleic acid predominated. The leaf oil of *Athrotaxis selaginoides* contained an unusually high amount (37%) of pentadecatrienoic acid, confirmed by retention times on GLC and by hydrogenation.

ACKNOWLEDGEMENT

Mr. K.J. Shaw measured the mass spectra of the methyl esters.

REFERENCES

1. Berry, S.K., J. Sci. Food Agric. 31:657 (1980).
2. Takagi, T., and Y. Itabashi, Lipids 17:716 (1982).
3. Schlenk, H., and J.L. Gellerman, JAOCS 42:504 (1965).
4. Kleiman, R., G.F. Spencer, F.R. Earle and I.A. Wolff, Chem. Ind. (London) 1326 (1967).
5. Jamieson, G.R., and E.H. Reid, Phytochemistry 11:269 (1972).
6. Vickery, J.R., JAOCS 57:87 (1980).
7. Vickery, J.R., JAOCS 58:731 (1981).
8. Mikolajczak, K.L., and M.O. Bagby, JAOCS 42:43 (1965).
9. Ford, G.L., F.B. Whitfield and K.H. Walker, Lipids 18:103 (1983).
10. Tinoco, J., and P.G. Miljanich, Anal. Biochem. 11:548 (1965).
11. Jackman, L.M., and S. Sternhell in Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, edited by D.H.R. Barton and W. Doering, Pergamon Press, London, 1969.
12. Levy, G.C., and G.L. Nelson, Carbon-13 Nuclear Magnetic Resonance for Organic Chemists, Wiley-Interscience, New York, 1972.

[Received July 26, 1983]