A Naturally Occurring All-*cis* 6,9,12,15-Octadecatetraenoic Acid in Plant Oils[1,2]

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Abstract

Oil extracted from freshly ground seed of *Onosmodium occidentale* contains 9-oleic, 9,12-linoleic, 6,9,12-linolenic, 9,12,15-linolenic, 6,9,12,15-octadecatetraenoic and 11-eicosenoic as unsaturated fatty acids. Column chromatography employing silicic acid-silver nitrate, and oxidation with permanganate-periodate was used in conjunction with GLC to confirm the structures of the unsaturated acids. Oils extracted from other members of the Boraginaceae family also contain the 6,9,12, linolenic and octadecatetraenoic acids.

Introduction

THE PRESENCE of some of the isomeric polyunsaturated fatty acids in fats and oils is readily detected in GLC analyses by comparing the emergence times with the commonly occurring acids on polar polyester columns (1,10). Oil extracted from the seed of *Onosmodium occidentale* was converted to methyl esters and analyzed by GLC using the *o*-phthalic ethylene glycol polyester. The GLC chart showed oleic, linoleic, and linolenic esters with emergence times coincident with known standards. Two additional peaks were found which appeared to be unusual component fatty acids. The composition of this oil was previously reported by Wolff et al. (5) in terms of saturates, monoenes, dienes, and trienes as a part of a routine survey. The presence of a nonconjugated tetraene in Anchusa capensis was reported by Earle et al. in an earlier paper (6).

Materials and Methods

Fresh, sound seed of *Onosmodium occidentale* was ground and extracted with Skellysolve F in Swedish extraction tubes (14). The clear yellow oil had an iodine value (I.V.) of 199.4. A sample of the oil (0.90 g) was saponified and the solution of soaps was shaken in a separatory funnel with ethyl ether to remove the unsaponifiable material (7). The soaps were acidified, extracted into ethyl ether, and converted to methyl esters using methanol and boron trifluoride as a catalyst (11). [The procedure of Metcalfe was modified. Boron trifluoride-etherate complex (1–3 drops) was added to 10–15 ml of methanol to prepare the esterification mixture.] The methyl esters had an I.V. of 198.4.

A conventional GLC isothermal unit using T/C detectors (3) was used with non-polar and polar columns. A 4 ft x ¼ in. copper column containing SE-30-Celite 1:6 w/w, 60–80 mesh, operated at 220C and 60 ml/min of helium was used for separation by chain length. An 8 ft x 3/16 in. copper column filled with *o*-phthalic-ethylene glycol polyester (2) on acid washed C-22 firebrick 1:4.5 w/w, 40–60 mesh was operated at 205C, 60 ml/min of helium for separation of individual fatty acid esters.

The permanganate-periodate oxidation procedure of von Rudloff (15,16) was used to determine the double bond positions in the unsaturated fatty acids. Monocarboxylic acids produced by the oxidative fission were analyzed as the decyl esters on the silicone column (4), and the dicarboxylic and long chain saturated acids were measured as methyl esters on the polyester column.

Column chromatography, using silicic acid-silver nitrate according to De Vries (17,18), was employed for separation of methyl esters of the fatty acids into classes according to degree of unsaturation. The separation of 120 mg esters was carried out using 14 g adsorbent in a column 1.5 cm diam x 23.0 cm long. Saturated acids were eluted with 10% benzene in Skellysolve F; monoethenoid with 25% benzene in Skellysolve F; diethenoid with 50–60% benzene in Skellysolve F; triethenoid with 5% ethyl ether in benzene, and tetraethenoid with ethyl ether.

UV spectral analyses were carried out according to the standard AOCS procedure (12), and IR analyses were made with a Perkin-Elmer Model 21 spectrometer.

I.V. was determined by the standard procedure (12) using 1 hr reaction time.

Results

GLC separation using the polyester column is shown in Figure 1. Emergence times for peaks 1,2,3, 4,6, and 8 were coincident with the more common fatty acids, whereas peaks 5 and 7 were unknown. The results from the non-polar SE-30 column showed

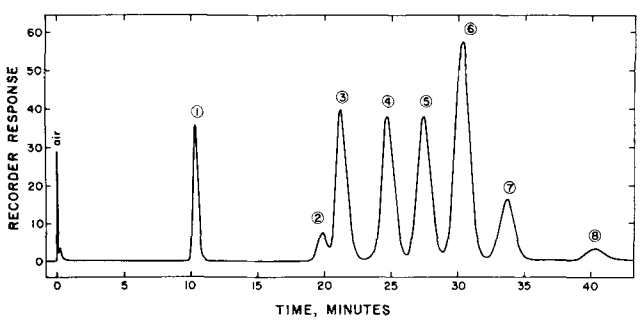

FIG. 1. GLC separation of methyl esters from oil of *Onosmodium occidentale* using an *o*-phthalic-ethylene glycol polyester. 1) palmitic; 2) stearic; 3) 9-oleic; 4) 9,12 linoleic; 5) 6,9,12-linolenic; 6) 9,12,15-linolenic; 7) 6,9,12,15-octodecatetraenoic; and 8) 11-eicosenoic.

TABLE I

GLC Analysis of Methyl Esters Using *o*-Phthalic-Ethylene Glycol Polyester

Peak number[a]	Fatty acid	% Composition
1	16:0	6.6
2	18:0	2.4
3	18:1	15.5
4	18:2	17.0
5	X	18.3
6	18:3	30.4
7	Y	8.2
8	20:1	1.6

[a] Peak numbers from GLC chart (Fig. 1).
GLC analysis of methyl esters on silicone column: C_{18}, 6.4%; C_{18}, 91.1%; and C_{20}, 2.5%.
GLC analyses of hydrogenated methyl esters on polyester column: 16:0, 6.6%; 18:0, 91.1%; and 20:0, 1.9%.
I.V.—calculated from GLC analyses 199.4; found 198.4.

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[2] Presented at the AOCS Meeting in Minneapolis, 1963.
[3] NRC of Canada Postdoctoarte Fellow, 1962.

TABLE II

Fission Products from $KMnO_4$–$NaIO_4$ Oxidation of Mixed Esters

	Number of C atoms in fatty acid								16:0	18:0
	3	4	5	6	7	8	9	11		
Dicarboxylic										
Found	.....	....	1.0	24.4	0.9	1.7	59.8	3.0	6.9	2.3
Expected	.....	....	....	26.3	....	....	62.1	1.6	7.3	2.7
Monocarboxylic										
Found	39.7	0.5	0.7	36.2	1.0	....	21.9			
Expected	42.5	....	....	38.6	....	....	18.9			

only three chain lengths; a comparison of the quantitative data obtained on the polyester column showed that peaks 5 and 7 belonged to the C_{18} series. Analyses of the hydrogenated sample provided further confirmation (Table I).

UV analyses by standard procedure (12) showed nonconjugated diene, triene, and tetraene in samples of methyl esters and fatty acids. The calculated I.V., based on the assumption of peak 5 as triene and 7 as tetraene, showed good agreement with the measured I.V.

The structure of the unsaturated fatty acids was first determined by oxidation of the mixed methyl esters with permanganate-periodate (15,16). The oxidation products showed major proportions of C_6 and C_9 and a minor proportion of C_{11} dicarboxylic acids (Table II). Since the molar amounts of 9-oleic, 9,12-linoleic and 9,12,15-linolenic could be accounted for by the C_9 dicarboxylic acid formed, and the amount of 11-eicosenoic by the C_{11} dicarboxylic acid, the two unknown acids must have ethylenic systems starting at the 6 position. If these acids are assumed to be 6,9,12-linolenic and 6,9,12,15-tetraenoic, reasonable agreement is obtained on the basis of found and expected values for the dicarboxylic acids. The proportions of monocarboxylic acids obtained in the oxidation afford confirmation of the assigned structures (Table II).

Further data were obtained by column chromatography using silicic acid-silver nitrate according to De Vries (17,18). The mixed esters were separated into the following fractions which were analyzed by GLC using the polyester column:

1. Saturated — 14:0, 0.3; 16:0, 73.2; 17:0, 0.8; 18:0, 25.7
2. Monoene — 18:1, 86.0; 20:1, 14.0
3. Diene — 18:2, 100.0
4. Triene — 18:3, 35.5; 18:3, 64.5
5. Tetraene — 18:4, 100.0

Each unsaturated fraction was then oxidized with permanganate-periodate reagent and the results (Table III) confirmed the structures assigned to the fatty acids on the basis of oxidation of the mixed esters.

The unique presence of the octadecatetraenoic acid in the *Onosmodium occidentale* was followed by the analysis of oils from other members of the Boraginaceae family (Table IV). In these cases the presence of the acids is assumed on the basis of equivalent emergence time to the methyl esters of *Onosmodium occidentale*.

TABLE III

Analyses of Fission Products from Fractions by Silicic Acid-Silver Nitrate Column Chromatography

	Fraction							
	II monoene		III diene		IV triene		V tetraene	
	Found	Expected	Found	Expected	Found	Expected	Found	Expected
Dicarboxylic								
5	0.6							
6	1.1		3.3		35.5	37.6	100	100
7	1.8		2.1					
8	2.2		3.5					
9	80.3	89.0	91.1	100	64.5	62.4		
11	14.0	11.0						
Monocarboxylic								
3	....		.....		64.7	64.4	100	100
4	....		1.7					
5	....		2.6					
6	....		95.7	100	35.3	37.6		
7	3.8		....					
8			....					
9	96.2	100						

Discussion

The isolation of the 6,9,12,15-octadecatetraenoic acid from vegetable oils has not been shown previously, although it does occur in fish oils (8,9). As noted in Table IV, the oils from four of the five members of the Boraginaceae family contain this fatty acid, with the oil from *Lappula echnata* having the highest proportion. It is also interesting to find that four members have oil containing both the isomeric linolenic acids, with Borage as an unusual system having no tetraene and only the 6,9,12-linolenic acid.

The use of the silicic acid-silver nitrate column chromatography (17,18) provides a valuable adjunct to GLC analysis. Roberts et al. (13) made use of this technique to demonstrate the presence of 6,9,12-linolenic acid in hop oil. The use of this technique showed the presence of small amounts of the odd numbered saturated acids which were not obvious on the GLC charts of the original ester mixture. The enrichment due to column chromatography providing a sharp separation on the basis of unsaturation, clearly demonstrates the advantage of this technique. It should be very useful in the analysis of complex oils such as butter, fish oils, and animal fats. It is possible to separate isomeric polyunsaturated acids such as the 6,9,12- and 9,12,15-linolenic acids by this system using benzene only as the eluting solvent.

Data in Table III indicate that some 11-oleic acid is present in the monoene fraction besides the 9-oleic acid. Oxidation data indicate that the eicosenoic acid is the 11-eicosenoic. The difference between the expected and found values for C_{11} dicarboxylic acid based on the proportions of C_{18} and C_{20} monoenes (3%) is balanced by 3.8% of C_7 monocarboxylic acid which must have resulted from oxidative scission of 11-oleic acid.

Further work is being done on oils from the members of the Boraginaceae family, to establish the extent to which the two isomeric families, e.g., 6- and 9-, exist in each class of unsaturated fatty acid.

TABLE IV

Fatty Acid Compositions of Some Members of the Boraginaceae Family

	% Oil	Fatty acid									
		16:0	16:1	18:0	18:1	18:2	18:3[a]	18:3[b]	18:4	20:1	22:1
1) *Onosmodium occidentale*	18	6.6	....	2.4	15.5	17.0	18.3	30.4	8.2	1.6	
2) *Myosotis arvensis*	32	9.1	0.3	2.8	28.8	27.4	6.9	8.6	6.7	5.0	4.4
3) *Lappula echinata*	25	6.0	0.3	1.8	12.9	14.9	8.6	35.2	18.6	1.7	....
4) *Borago officinalis*	35	11.7	0.4	4.4	18.4	37.9	20.7	.....	.....	3.9	2.6
5) *Anchusa azurea*	25	7.9	0.4	1.5	22.4	31.8	9.1	17.9	3.5	3.3	2.2

[a] 6,9,12-linolenic acid.
[b] 9,12,15-linolenic acid

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Determination of Residual Solvents in Solvent Extracted Meals[1]

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A REVIEW OF THE literature revealed that only a few methods have been developed for the estimation of residual solvents in extracted materials. A modified Pensky-Martens closed-cup flash tester (1) has been used for meal samples. A copper cup flash tester with concentric rings as heating surfaces is claimed (2) to detect solvent residues as low as 0.03%. The lowest flash temp is related to the solvent percentage in the meal. However, while testing this apparatus we found that, at the lower solvent levels (0.03–0.06%), it was difficult to detect "pop" noises or flashes when a test flame was applied at the appropriate temperature.

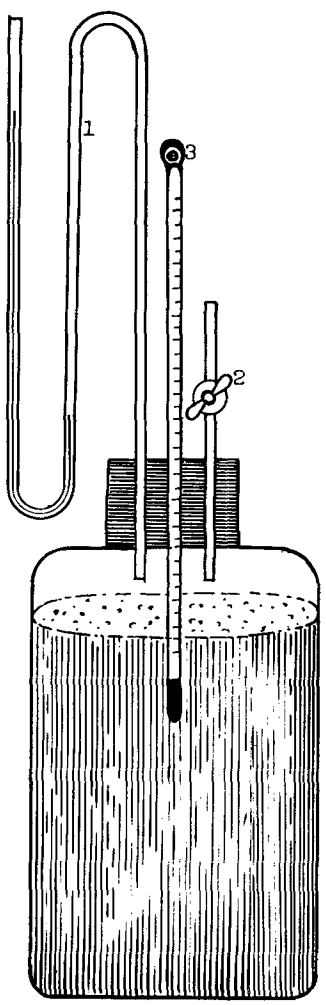

FIG. 1. Apparatus for measurement of solvent residues.

In the course of our work on the solvent extraction of edible quality meals, it became necessary to have a quick method to determine the residual solvent in the extracted meals. A method which we found to be useful is based on vapor pressure of the solvent:

The apparatus consists of a glass bottle which can hold about 300 g meal (Fig. 1), with a tight-fitting rubber stopper carrying a capillary water manometer with scale, a stopcock, and a thermometer.

Meal samples of known solvent content were prepared by a method similar to that of Gastrock et al. (2). The peanut meal used contained 1.5% residual oil and 6% H_2O. About 100 g of solvent-free meal were placed in the bottle and the required amount of solvent hexane or heptane added by pipette. The rest of the meal was then quickly added and the stopper fitted tightly. The stopcock, which was open in the beginning, was now closed. After allowing 1 hr for equilibration at the room temp, the pressure recorded by the manometer was noted.

The pressures recorded by the manometer for various hexane and heptane percentages in the meal are shown in Figure 2. The lowest hexane percentage which gave measurable pressure was 0.04%. Gas-

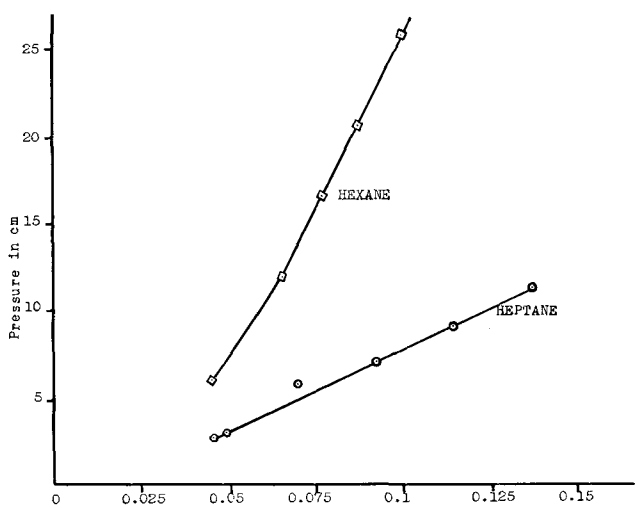

FIG. 2. Per cent solvent in meal.

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