Nonpolar Lipids of *Amaranthus palmeri* S. Wats. 2. Unsaturated Esters and Free Fatty Acids, Sterols, and Triterpenols

Oliver D. Dailey, Jr.,*,† Ray F. Severson,‡,§ and Richard F. Arrendale‡,||

Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 19687, New Orleans, Louisiana 70179, and Richard B. Russell Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 5677, Athens, Georgia 30613

An earlier paper on the nonpolar lipids of *Amaranthus palmeri* described the compositions of the saturated fatty alcohol and wax ester components of the leaves and flowering parts. In this paper, the compositions of esters containing unsaturated acyl moieties (triglycerides, steryl esters, and terpenol esters), as determined by capillary gas chromatography/mass spectroscopy (GC/MS) analysis, are presented. In addition, the free fatty acid, sterol, and triterpenol components were characterized. The triglycerides constituted the major class of esters. The major hydroxylated constituents were palmitic, linoleic, and oleic acids and the sterol chondrillasterol. The sterols campesterol, stigmasterol, ergost-7-en-3β-ol, chondrillast-7-enol, and 24-ethylidenecholest-7-en-3β-ol and the triterpenols β-and α-amyrin, lupeol, cycloartenol, and 24-methylenecycloartenol were present in lesser quantities.

Keywords: *Lipid; Amaranthus palmeri; fatty acid; sterol; triterpenol; column chromatography; thin-layer chromatography; capillary gas chromatography; GC/MS*

INTRODUCTION

Palmer amaranth (*Amaranthus palmeri* S. Wats) is a common agronomically significant weed, the soil-incorporated residues of which have been observed to inhibit the growth of certain crop plants, most notably carrot and onion. The weed residues are also autotoxic (Menges, 1987). In their search for allelopathic agents from *A. palmeri*, Fischer and Quijano (1985) isolated phytol and chondrillasterol from a petroleum ether extract of aerial parts. They proposed a mechanism by which water-insoluble plant lipids such as sterols may act as allelochemicals.

There have been a number of investigations of the composition of the seed lipids of both vegetable amaranths [*Amaranthus caudatus* (*A. edulis*), *A. cruentus*, *A. dubius*, *A. tricolor* (*A. gangeticus*)] and weedy species (*A. hybridus*, *A. retroflexus*, *A. spinosus*) (Becker et al., 1981; Dixit and Varma, 1971; Fernando and Bean, 1985; Bressani et al., 1987; Lorenz and Hwang, 1985; Opute, 1979; Stoller and Weber, 1970; Prakash and Pal, 1992). All of the investigators determined the major fatty acids of the seeds to be linoleic (C-18:2), oleic (C-18:1), stearic (C-18:0), and palmitic (C-16:0). The unsaturated acids constituted about 70% of the total acids. Trace amounts of the following acids were found in some of the studies: myristic (C-14:0); palmitoleic (C-16:1), linolenic (C-18:3), arachidic (C-20:0), and lignoceric (C-24:0). In one study (Fernando and Bean, 1985), the fatty acid compositions of vegetable and weedy species were virtually the same.

In three of the aforementioned studies (Lorenz and Hwang, 1985; Opute, 1979; Stoller and Weber, 1970), the nonpolar lipids were further characterized as triglycerides (major component), sterols, sterol esters, diglycerides, monoglycerides, free fatty acids, and hydrocarbons. Dixit and Varma (1971) isolated β-sitosterol from the seeds of *A. caudatus*. The following sterols were found in the seeds of *A. tricolor*, *A. retroflexus*, *A. hybridus*, *A. dubius*, and *A. cruentus*: spinasterol, Δ^7-stigmasterol, Δ^7-ergosterol, stigmasterol, and 24-methylenecycloartenol (Fernando and Bean, 1985).

Lakshminarayana et al. (1984) published a comprehensive report of the lipid and fatty acid composition of the leaves of young *A. gangeticus* (*A. tricolor*) plants. The following nonpolar lipids were analyzed for fatty acid composition: ester waxes, fatty acid methyl esters, triglycerides, free fatty acids, diglycerides, and monoglycerides.

In an earlier paper on the nonpolar lipids of *A. palmeri* (Dailey et al., 1989), we described the compositions of the saturated fatty alcohol and wax ester components of the leaves and thyrses. In this paper, the composition of esters containing unsaturated acyl moieties (triglycerides, steryl esters, and terpenol esters), as determined by capillary gas chromatography/mass spectroscopy (GC/MS) analysis, will be presented. In addition, the free fatty acid, sterol, and triterpenol components will be discussed.

MATERIALS AND METHODS

Reagents. Boron trichloride/methanol, *N*,*O*-bis(trimethylsilyl)acetamide (BSA), bis(trimethylsilyl)trifluoroacetamide (BSTFA), and dimethylformamide (DMF) were purchased from Pierce Chemical Co., Rockford, IL. Hexanes and diethyl ether (ether) were purchased as Baker Analyzed reagents from J. T. Baker Chemical Co., Phillipsburg, NJ. All extractions and chromatography using these solvents were performed in a hood.

Extraction of Plant Material. *A. palmeri* plant material was obtained, processed, and extracted as previously described (Dailey et al., 1989). Extraction of 1681 g of ground leaves and thyrses with hexanes (a mixture of isomers) furnished 11.2 g of extract **A** (Figure 1).

* Author to whom correspondence should be addressed [e-mail odailey@nola.srrc.usda.gov; fax (504) 286-4367].

† Southern Regional Research Center.

‡ Richard B. Russell Agricultural Research Center.

§ Deceased, April 12, 1994.

|| Present address: Solvay Pharmaceuticals, 901 Sawyer Rd., Marietta, GA 30062.

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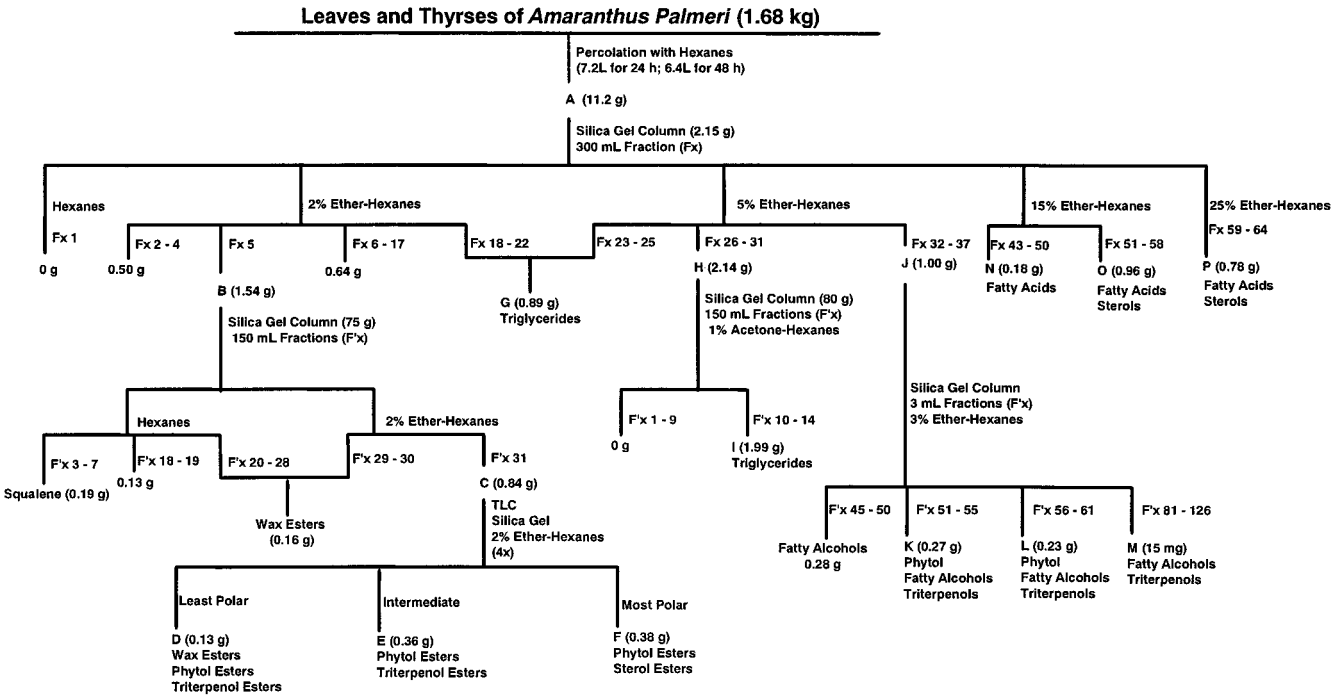

Figure 1. Scheme for the extraction and isolation of the nonpolar lipids from *A. palmeri* leaves and thyrses.

Table 1. Relative Distributions of Nonpolar Lipids of *A. palmeri* for the Original Hexane Extract A and Derived Fractions[a]

relative % distribution		original extract	free alcohols and triterpenols			free fatty acids and sterols		hydrolysis product from		
Rt	compound	**A**	**K**	**L**	**M**	**O**	**P**	**F**	**E**	**D**
17.6	palmitic acid	76			55	47	42			
19.6	phytol		100	66				56	90	100
20.1	linoleic acid	100			4	54				
20.2	oleic acid	96			8	44	100			
20.6	stearic acid	12			12	12	8			
22.2	1-eicosanol	<1	3	3	2			1	4	4
23.4	arachidic acid	4				3				
24.8	1-docosanol	2	18	25	6			2	10	10
26.0	docosanoic acid	5				5	2			
26.1	1-tricosanol		3	7						
27.3	1-tetracosanol	2	70	83	66			9	26	25
28.4	lignoceric acid	1				7	3			
28.5	1-pentacosanol		3	4	7					
29.1	*n*-nonacosane	9								
29.6	1-hexacosanol	2	24	43	36			4	10	10
30.7	1-heptacosanol		2	4	4					
30.8	hexacosanoic acid	8				2	<1			
31.4	*n*-hentriacontane	11								
31.8	heptacosanoic acid					1				
31.9	1-octacosanol	8	56	100	100			3	23	22
32.9	1-nonacosanol		4	7	10					
33.0	octacosanoic acid and campesterol	2				8	3	10		
33.4	stigmasterol	1				3		4		
33.5	stigmasta-22-en-3β-ol (a)	6				14	1	4		
33.7	ergost-7-en-3β-ol (b)	4				14	7	99		
33.9	β-amyrin	8	49	63	81				100	23
34.1	1-tricontanol		29	63	2				9	53 (g)
34.1	chondrillasterol (c)	52 (e)				100	57	100		
34.4	α-amyrin	1	19	25					4	12
34.5	lupeol	2	21	40					9	1
34.6	cycloartenol (d)		6	6					38	1
34.7	chondrillast-7-enol (c)	5 (f)				18	7	85		
34.8	24-ethylidenecholest-7-en-3β-ol (b)	<1				3	2	44		
34.9	1-hentriacontanol		3	4						
35.2	24-methylenecycloartenol (d)	2	38	25					10	<1
35.9	1-dotriacontanol	2	6	11	4				6	24 (g)

[a] Comparison with authentic standards used for identifications, except the ones noted as follows: (a) tentative identification from interpretation of mass spectrum; (b) comparison with reference spectra (Stenhagen et al., 1974); (c) comparison with reference spectra supplied by Dr. N. H. Fischer, Department of Chemistry, LSU, Baton Rouge, LA; (d) GC retention and GC/MS data consistent with that reported by Severson et al. (1978); (e) coelution of 1-tricontanol and chondrillasterol; (f) coelution of cycloartenol and chondrillast-7-enol; (g) coelution with unknown compound.

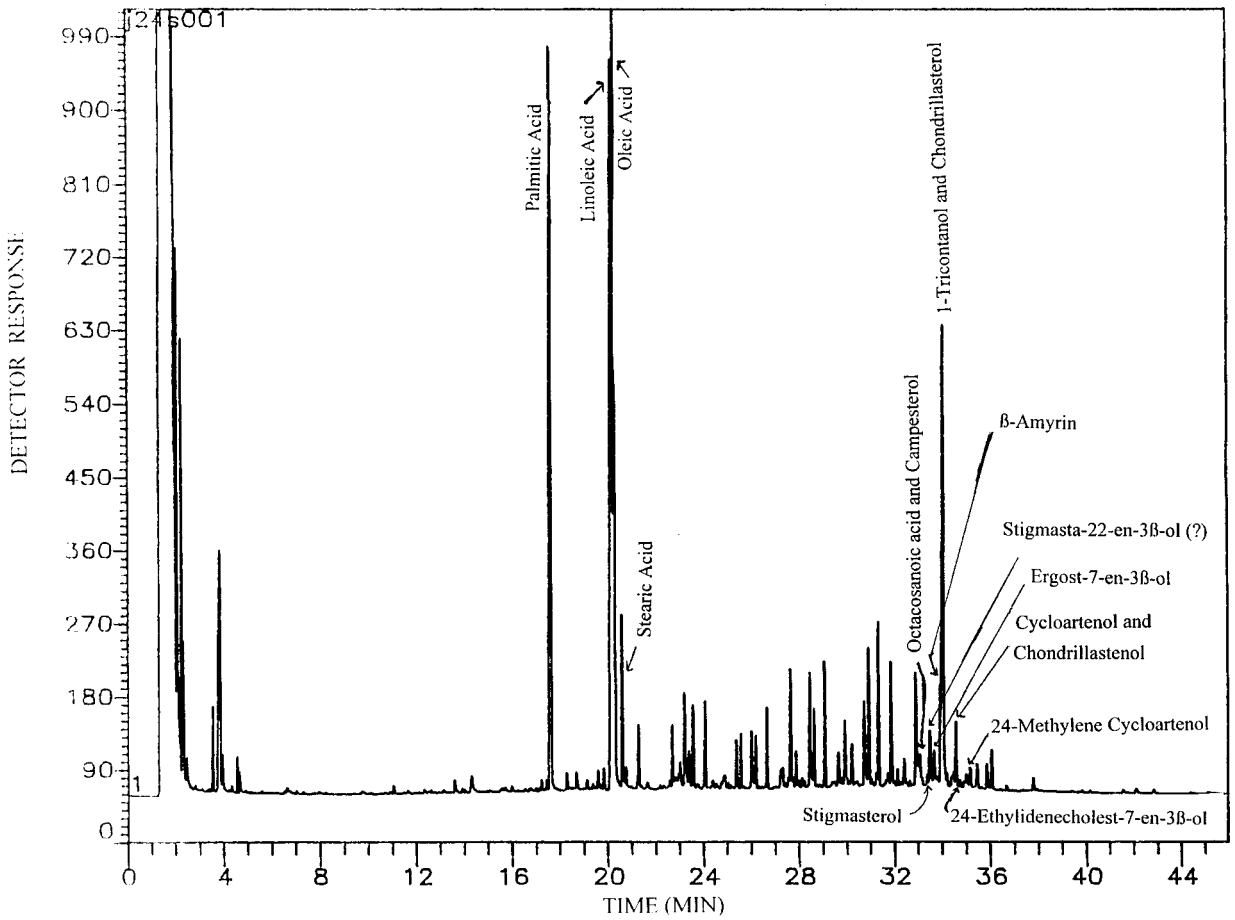

Figure 2. Capillary gas chromatogram of fraction **A** (analyzed as trimethylsilyl derivatives).

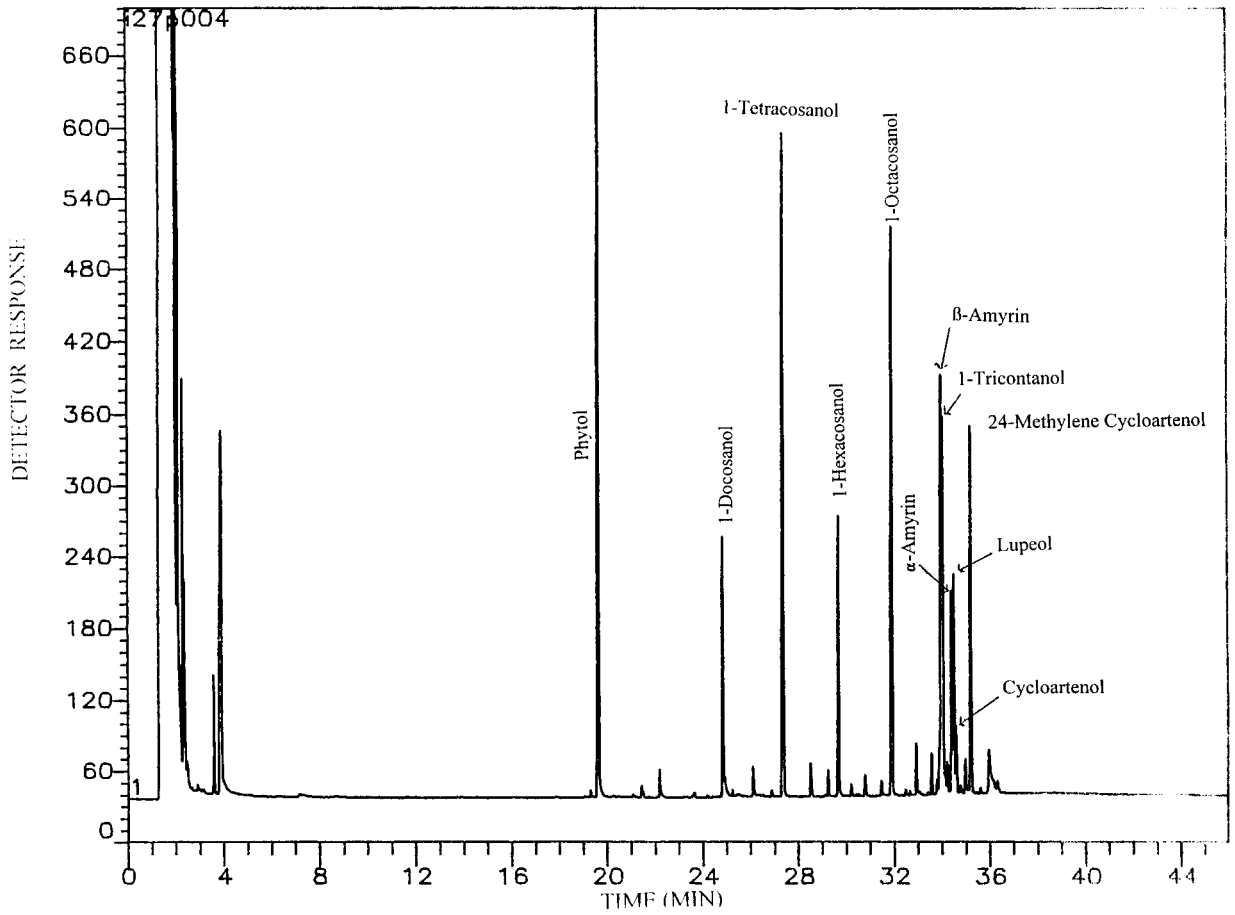

Figure 3. Capillary gas chromatogram of fraction **K** (analyzed as trimethylsilyl derivatives).

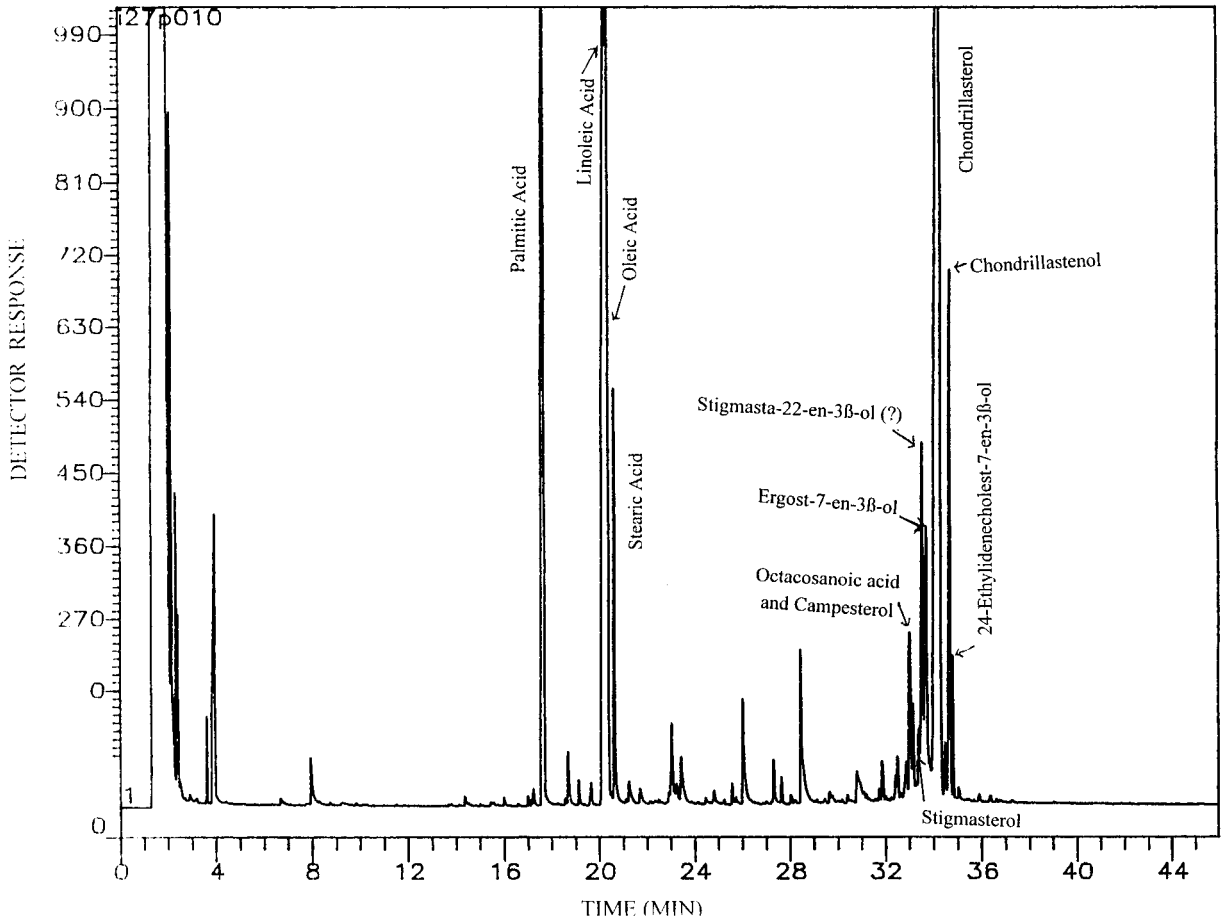

Figure 4. Capillary gas chromatogram of fraction **O** (analyzed as trimethylsilyl derivatives).

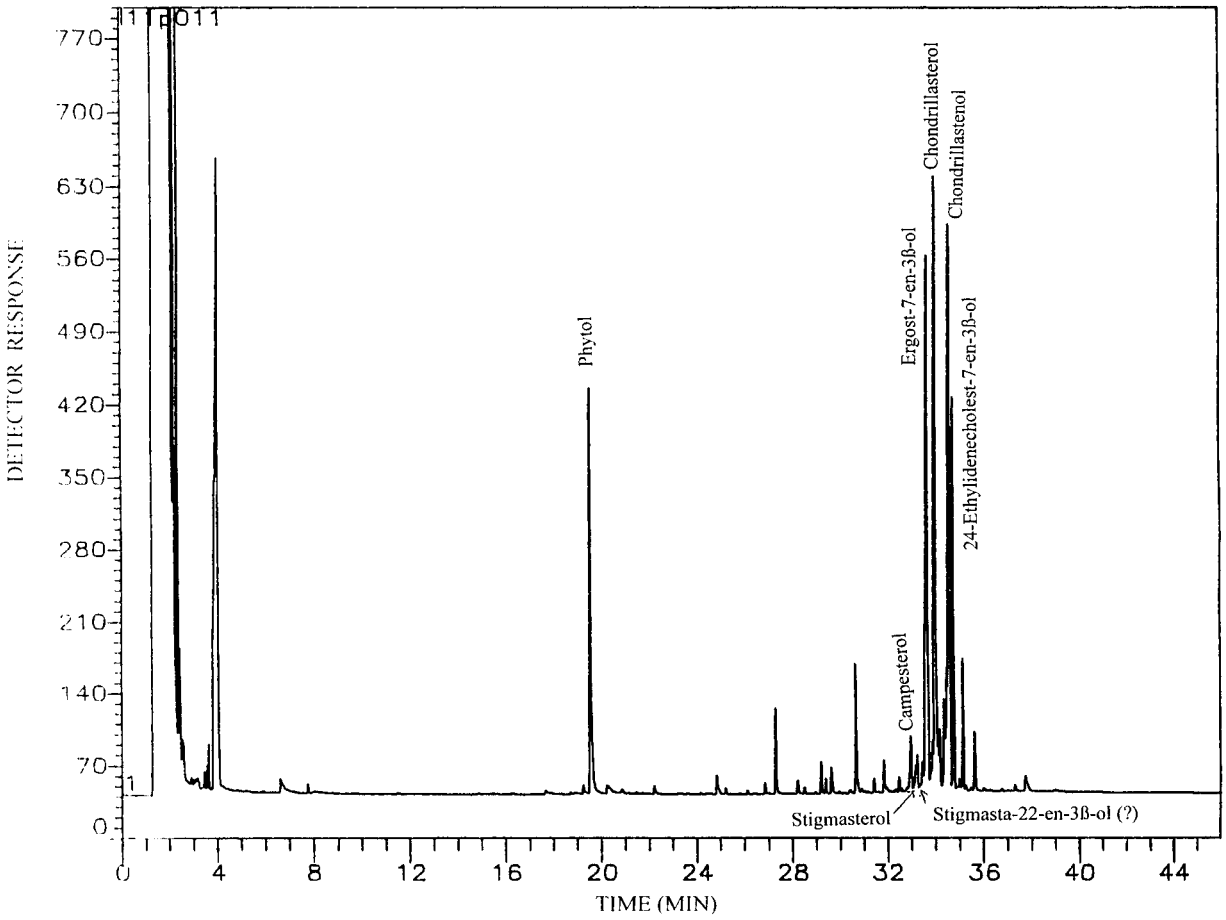

Figure 5. Capillary gas chromatogram of the alcohol moieties of fraction **F** (analyzed as trimethylsilyl derivatives).

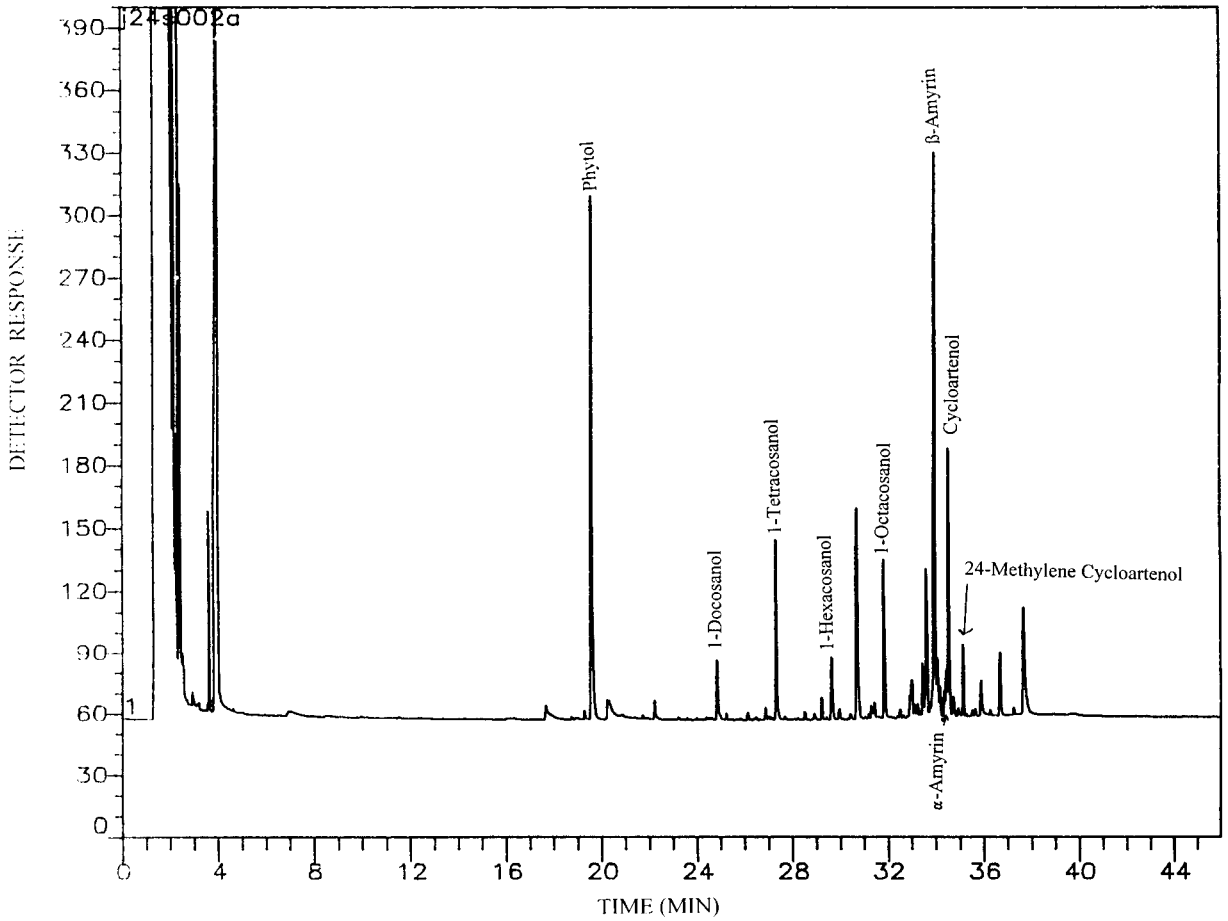

Figure 6. Capillary gas chromatogram of the alcohol moieties of fraction **E** (analyzed as trimethylsilyl derivatives).

Table 2. Relative Levels[a] of Fatty Acid Moieties in Unsaturated Ester Fractions[b]

	ester fraction				
fatty acid	**D**	**E**	**F**	**G**	**I**
C-16:0	100	100	100	100	100
C-18:0	17	38	3	15	2
C-18:1	43	81	122	75	111
C-18:2	23	187	863	153	395
C-18:3	7	106	421	1	5
C-20:0	10	7	—[c]	5	1
C-22:0	12	5	—	3	—
C-24:0	9	4	—	2	—
C-26:0	2	—	—	—	—

[a] [Amount of acid X/amount of C-16:0] × 100. [b] Analyzed as methyl esters after transesterification of ester fraction with boron trichloride/methanol. [c] Absent below 1%.

Fractionation of the Hexane Extract A. As shown in Figure 1, extract **A** (10.6 g of 11.2 g isolated) was chromatographed on a silica gel column by eluting first with hexanes and then with increasing percentages of ether in hexanes (300-mL fractions). Fraction (fx) 5 was eluted with 2% ether/hexanes and afforded 1.54 g of **B**. Subsequent elution with 2% ether/hexanes (fx 18−22) and 5% ether/hexanes (fx 23−25) provided 0.89 g of **G**. Continued elution with 5% ether/hexanes gave 2.14 g of **H** (fx 26−31), 1.00 g of **J** (fx 32−37), and 0.34 g of a complex mixture not studied futher (fx 38−42). Continued elution with solvent mixtures ranging from 15% ether/hexanes to 25% ether/hexanes furnished fractions **N**, **O**, and **P**. An additional 0.61 g of material was eluted with 25% ether/hexanes, 50% ether/hexanes, and ether. In all, 9.40 g of material was eluted from the column.

As shown in Figure 1, fraction **B** was rechromatographed on a silica gel column. The characterization of fx 3−7 and 20−30 has been discussed previously (Dailey et al., 1989). Subfraction 31 afforded 844 mg of **C**, which was subjected to preparative thin-layer chromatography on eight 20 cm × 20 cm × 2 mm silica gel plates. The plates were each fully developed four times with 2% ether/hexanes to effect complete separation of the major bands. The plates were dried completely after each development. Extraction of the three distinct bands thus obtained with 20% ether/hexanes yielded 376 mg of **F** (most polar band), 360 mg of **E** (intermediate), and 126 mg of **D** (least polar). Fraction **H** was further purified on a silica gel column to furnish 1.99 g of **I**. Finally, **J** was chromatographed on a second column (Figure 1), providing **K**, **L**, and **M**. The composition of subfractions 45−50 (fatty alcohols) has been discussed (Dailey et al., 1989).

Hydrolysis of Ester Fractions D−G and I. For acid analysis, 0.5−1.0 mg of ester was heated in 3 mL of boron trichloride/methanol for 3 h at 76 °C. The mixture was cooled, 1 mL of water was added, and the methyl esters were extracted with *n*-hexane (2 mL, 1 mL, 1 mL).

GC/Mass Spectrometry. Aliquots from fractions **A**, **D**−**F**, **K**−**M**, **O**, and **P** were treated with a 1:1 mixture of BSTFA−DMF for 30 min at 76 °C to derivatize the hydroxylated components to trimethylsilyl (TMS) ethers or esters. Low-resolution electron impact (EI) GC/MS data were obtained on a Hewlett-Packard 5985B, modified for capillary GC/MS application (Arrendale et al., 1984), equipped with a 30 m × 0.32 mm i.d. thin film (about 0.1 μm) SE-54 fused silica capillary column which was coated in-house (Martin et al., 1985) and a splitless injection port (250 °C). The following temperature program was used: 100 °C, 2 min hold, 4 °C/min to 260 °C, then 3 °C/min to 310 °C. The carrier gas was helium, and the flow rate was 40 cm/s. A source temperature of 200 °C and an electron energy of 70 eV, scanning from 40 to 700 amu with electron multiplier voltage of 1800 V, were used for these analyses.

Capillary Gas Chromatography (GC-2). For original samples **D**−**G** and **I** (esters), initial capillary gas chromatography studies (before hydrolysis) were conducted on a Hewlett-Packard 5890 equipped with a 9 m × 0.5 mm i.d. SE-54 fused

silica column (0.25 μm film thickness, coated in-house), splitless injection port (250 °C), and flame ionization detector (325 °C) using helium carrier gas (25 mL/min). The following temperature program was used: 100 °C for 2 min to 310 °C at 10 °C/min; hold at 310 °C for 10 min.

In preliminary studies, free alcohols (sterols) were converted to their TMS ethers by treatment with BSA at 76 °C for 30 min or by treatment with BSTFA. The TMS derivatives were analyzed on a HP 5720 GC equipped with a 30 m × 0.32 mm i.d. thin film (about 0.1 μm) SE-54 fused silica column (Martin et al., 1985), splitless injection port (250 °C), and flame ionization detector (325 °C) using helium carrier gas at a flow rate of 40 cm/s. The column temperature was programmed at 100 °C for 2 min to 330 °C at 5 °C/min.

The relative distribution table (Table 1) for the original extract **A** and derived fractions was developed from GC/FID data. Analyses of TMS derivatives of constituent acids and alcohols were conducted on a Hewlett-Packard 5890 equipped with a 30 m × 0.32 mm i.d. thin film (about 0.1 μm) SE-54 fused silica capillary column, splitless injection port (250 °C), and flame ionization detector (320 °C), using hydrogen carrier gas (approximately 50 cm/s linear velocity). The following temperature program was used: 100 °C, 1 min hold, 6 °C/min to 320 °C, 15 min hold.

The fatty acid methyl esters were analyzed on a 15 m × 0.32 i.d. Carbowax 20M3NPA column (film thickness about 0.1 μm, coated in-house) using helium carrier gas (5 psi). The column temperature was programmed at 100 °C for 2 min to 220 °C at 3 °C/min. Peak areas were corrected for the difference in chromatographic response by conventional internal standard calibration methods and authentic standards. The data obtained from these analyses are reported in Table 2.

RESULTS AND DISCUSSION

The relative distributions of the nonpolar lipids of *A. palmeri* for the original hexane extract **A** and derived fractions **K**−**M** (free alcohols and triterpenols) and **O** and **P** (free fatty acids and sterols) are given in Table 1. All fractions were analyzed as TMS derivatives of the constituent acids and alcohols. The gas chromatogram of **A** is shown in Figure 2. The major constituents are palmitic, linoleic, and oleic acids and the sterol chondrillasterol (coeluted with 1-tricontanol). The sterols campesterol, stigmasterol, ergost-7-en-3β-ol, chondrillastenol, 24-ethylidenecholest-7-en-3β-ol, and possibly stigmasta-22-en-3β-ol and the triterpenols β- and α-amyrin, lupeol, cycloartenol, and 24-methylenecycloartenol are present in lesser quantities. The gas chromatogram of the free alcohol and triterpenol fraction **K** is shown in Figure 3, and that of the free fatty acid and sterol fraction **O** is shown in Figure 4.

Fraction **F** consisted almost exclusively of phytol C-18 esters and sterol esters. Fractions **E** and **D** contained phytol C-16 and C-18 esters, wax esters, and triterpenol esters. The gas chromatogram of the alcohol moieties of **F** (analyzed as TMS derivatives) is shown in Figure 5. The major constituents were phytol, ergost-7-en-3β-ol, chondrillasterol, chondrillast-7-enol, and 24-ethylidenecholest-7-en-3β-ol. The sterols stigmasterol, campesterol, and possibly stigmasta-22-en-3β-ol were present in small amounts. The gas chromatogram of the alcohol moieties of **E** (Figure 6) revealed the presence of phytol, the triterpenols β- and α-amyrin, lupeol, cycloartenol, and 22-methylenecycloartenol and substantial quantities of fatty alcohols. The major alcohol constituents of **D** were phytol, fatty alcohols, and β- and α-amyrin.

Stigmasterol and 24-methylenecycloartenol have been found in other *Amaranthus* species (Dixit and Varma, 1971; Fernando and Bean, 1985). Chondrillasterol has been previously isolated from the aerial parts of *A. palmeri* (Fischer and Quijano, 1985). The other sterols and triterpenols discussed above have not been found previously in *Amaranthus* species.

The gas chromatograms of **G** and **I** were very similar, both consisting primarily of three peaks representing three classes of triglycerides: those containing one C-18 and two C-16 acid moieties; those containing one C-16 and two C-18; and those containing three C-18 acid moieties. The major fatty acids of both **G** and **I** were linoleic (C-18:2), palmitic (C-16:0), and oleic (C-18:1) (Table 2). There were only small amounts of stearic (C-18:0) and linolenic (C-18:3) acids. In contrast, Lakshminarayana et al. (1984) found that the major fatty acid of the triglycerides of young *A. gangeticus* leaves was linolenic, followed by palmitic, palmitoleic (C-16:1), and linoleic.

Data on the relative distibution of the fatty acids in ester fractions **D**−**F**, as well as triglyceride fractions **G** and **I** are presented in Table 2. The major fatty acid constituents of fractions **E** and **F** were linoleic and linolenic, whereas the major acid of fraction **D** was palmitic. The most polar fraction, **F**, contained 93% unsaturated acids, fraction **E** contained 71%, and the least polar fraction, **D**, contained only 33%. Since sterols were the major alcohol moieties of **F**, they must exist primarily as esters of linoleic and linolenic acids.

CONCLUSIONS

In this paper, we have described the composition of a hexanes extract of the leaves and thyrses of *A. palmeri*. The following components were characterized: triglycerides, steryl esters, terpenol esters, free fatty acids, sterols, and triterpenols. The esters contained primarily unsaturated acyl moieties. The major acids were palmitic, linoleic, and oleic. The sterols chondrillasterol, campesterol, stigmasterol, ergost-7-en-3β-ol, chondrillast-7-enol, and 24-ethylidenecholest-7-en-3β-ol and the triterpenols β- and α-amyrin, lupeol, cycloartenol, and 24-methylenecycloartenol were present in both the free and ester form. Only chondrillasterol, stigmasterol, and 24-methylenecycloartenol had been identified in *Amaranthus* species previously.

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