High-Performance Liquid Chromatography of the Triacylglycerols of *Vernonia galamensis* and *Crepis alpina* Seed Oils

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The triacylglycerols of *Vernonia galamensis* and *Crepis alpina* seed oils were characterized because these oils have high concentrations of vernolic (*cis*-12,13-epoxy-*cis*-9-octadecenoic) and crepenynic (*cis*-9-octadecen-12-ynoic) acids, respectively. The triacylglycerols were separated from other components of crude oils by solid-phase extraction, followed by resolution and quantitation of the individual triacylglycerols by reversed-phase high-performance liquid chromatography with an acetonitrile/methylene chloride gradient and flame-ionization detection. Isolated triacylglycerols were characterized by proton and carbon nuclear magnetic resonance and by capillary gas chromatography of their fatty acid methyl esters. The locations of the fatty acids on the glycerol moieties in the oils were obtained by lipolysis. The *Vernonia galamensis* oil contained 50% trivernoloyl and 21% divernoloyllinoleoyl glycerols along with 20% triacylglycerols with one vernolic and two other fatty acids. The *Crepis alpina* oil contained 36% tricrepenynoyl and 33% dicrepenynoyllinoleoyl glycerols, 17% triacylglycerols with two crepenynic and one other fatty acid and 7% triacylglycerols with one crepenynic acid and two other fatty acids. Vernolic acid was found at both the 1(3)- and 2-glycerol carbons but was more abundant at the 1,(3)-position in the *Vernonia galamensis* oil. Crepenynic acid was found at both glycerol carbon positions but was more abundant at the 2-position in the *Crepis alpina* oil.

KEY WORDS: Carbon NMR, crepenynic acid, epoxy and acetylenic monoacid triglycerides, flame-ionization detection, gas chromatography, proton NMR, reversed-phase high-performance liquid chromatography, stereochemistry, vernolic acid.

Vernolic acid, *cis*-12,13-epoxy-*cis*-9-octadecenoic acid (Ve), and crepenynic acid, *cis*-9-octadecen-12-ynoic acid (Cr), may be obtained in 70–80% yield from *Vernonia galamensis* (VeGO) and *Crepis alpina* (CrAO) seed oils, respectively (1,2). These fatty acids (FA) have been used as precursors for preparation of deuterium-labeled methyl linoleate and its geometric isomers for metabolic studies of fats in humans (2). Vernolic acid can also be used in the manufacture of plastic formulations and paints (3–8). VeGO is under development as a potential oilseed crop and the processing of its oil has been investigated (3–8). Crepenynic acid from CrAO is potentially useful as an intermediate in chemical synthesis not only for deuterium-labeled compounds, but also for the production of conjugated triene intermediates (6).

Triacylglycerol (TAG) composition of VeGO obtained by gas-chromatographic analysis was reported by Carlson and Chang (4), whereas the TAG composition of CrAO has not been reported. Because these oils contain, respectively, reactive epoxy and acetylenic unsaturated FA, as well as other unsaturated FA, gas chromatography (GC) may not be entirely suitable for intact TAG analysis. For example, Christie (9) indicated that TAG with unsaturated FA may undergo possible thermal alteration due to the high temperature required for the GC analysis. Christie (9) reviewed methods for TAG analysis without thermal alteration by reversed-phase high-performance liquid chromatography (RP-HPLC) and concluded that the best HPLC method for characterizing unsaturated TAG is a mobile gradient of acetonitrile and methylene chloride, and a detector based on the transport flame-ionization principle (FID), which gives a quantitative response (10).

We report here a qualitative and quantitative TAG analysis of *Vernonia galamensis* and *Crepis alpina* by RP-HPLC-FID, as well as a direct stereospecific analyses of the TAGs of these oils.

EXPERIMENTAL PROCEDURES

Materials. VeGO and CrAO oils were obtained from Drs. K. Carlson and R. Kleiman (USDA, National Center for Agricultural Utilization Research, Peoria, IL). The oils had been extracted from seeds ground in a hammer mill, followed by hexane extraction of the ground material. The oils, stripped of hexane, had been stored at −30°C for two months. Solid-phase extraction columns (SE) (6.5 mL, 2.0 g silica) were used to remove non-TAG components from the crude oils and were purchased from Baxter Health Care (Muskegon, MI). SE columns ("Bond-Elut", 3 mL, 0.2 g silica) used for resolution of lipolysis mixtures were purchased from Analytichem International (Harbor City, CA). Pancreatic lipase (EC 3.1.1.3, Type 2, crude from porcine pancreas, activity 220 units per 1 mg protein with olive oil as substrate at pH 7.7), bile salts and sodium cholate were purchased from Sigma Chemical Company (St. Louis, MO). Thin-layer chromatography (TLC) plates (2.5 × 7.5 cm, 250 μm layer of Silica Gel A, ultraviolet 254 nM indicator) were obtained from Whatman Manufacturing (Fairfield, NJ). Reference standards were purchased from Nu-Chek-Prep, Inc. (Elysian, MN) for RP-HPLC-FID (Standard 50 A, Triglyceride Series) and for GC (Standard 15A, Methyl Ester Series). All solvents were HPLC-grade.

SE. To avoid non-TAG interference during RP-HPLC-FID of TAG mixtures, we chromatographed the crude oils by SE before HPLC analysis. The SE involved slurrying 35% by wt activated carbon (430 mg) and crude oil (1.20 g) in 1 mL hexane. This slurry was added to the top of the 6.5-mL SE column, which had been previously washed with hexane. Isolation of the VeGO TAGs was accomplished by elution with 1.5 mL hexane (fraction 1; 0.1 mg nonpolar material), 30 mL diethyl ether/hexane (1:1, vol/vol) (fraction 2; 1.1279 g TAG pure by TLC) and 30 mL methanol (fraction 3; 15.0 mg polar non-TAG components) from 1.2016 g crude oil. Solvents were removed by evaporation, and the fractions were analyzed by TLC (silica) with diethyl ether/hexane (1:1, vol/vol) as solvent and iodine vapor (I_2) visualization. R_f values were: fraction 1, 0.91; fraction 2, 0.76, 0.62; fraction 3, 0.43, 0.31, 0.13, 0.04, 0.0. Similarly, the CrAO TAGs were isolated by elution

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TABLE 1

Fatty Acid Compositions (capillary gas chromatographic[a] area percent) of the Triacylglycerols of Crude and Chromatographed[b] *Vernonia galamensis* and *Crepis alpina* Seed Oils

Fatty acid[d]	GC retention (min)	Area percent[c]			
		Vernonia galamensis oil		*Crepis alpina* oil	
		Crude	Chromatographed	Crude	Chromatographed
P	6.32 ± 0.05	3.2	3.1	3.8	4.1
S	9.47 ± 0.08	2.9	2.4	1.3	1.5
O	10.60 ± 0.00	4.8	4.2	2.5	2.9
L	12.46 ± 0.08	14.7	15.2	18.5	20.7
Cr	17.25 ± 0.01			73.9	70.8
Ve	26.03 ± 0.04	74.4	75.1		

[a,b]See Experimental Procedures section. GC, gas chromatography.
[c]SD = 0.1–0.5%.
[d]Cr, Crepenynic; Ve, vernolic; P, palmitic; S, stearic; O, oleic; L, linoleic.

with 1.5 mL hexane (fraction 1; 0.2 mg nonpolar material), 15 mL diethyl ether/hexane (10:90, vol/vol) (fraction 2; 1.0503 g TAG) and 15 mL methanol (fraction 3; 55.2 mg polar non-TAG components) from 1.2245 g crude oil. TLC R_f values: fraction 1, 0.82; fraction 2, 0.53; fraction 3, 0.29, 0.16, 0.07, 0.0.

Stereospecific analysis. Average compositions of FA on the 2-position and 1,(3)-position of the TAG were obtained by lipolysis and resolution of lipolysis mixture by SE chromatography as described previously (11). TLC (silica) analysis of lipolysis products with diethyl ether/hexane (60:40, vol/vol) as solvent and I_2 visualization yielded the following R_f values for VeGO: unreacted TAG, 0.78; FA, 0.52; diglyceride, 1,2;2,3, 0.30; 2-monoglycerol, 0.05; and for CrAO: unreacted TAG, 0.86; FA, 0.57–0.48; diglycerol, 0.42 and 2-monoglycerol, 0.07, 0.02. The FA compositions at the internal 2- and external 1,(3)-positions were calculated as reported previously (11).

RP-HPLC-FID. Analysis of TAG was performed in triplicate with 0.5 mg of purified TAG mixture dissolved in 5–10 μL hexane and injected onto two C-18 columns (5 micron, 0.49 × 50 cm, Zorbax, Dupont Inst. Div., Wilmington, DE) placed in series. The TAG were resolved with a 120-min gradient of 70:30 to 40:60 acetonitrile/methylene chloride (vol/vol) pumped at 0.5 mL/min. Columns were cleaned between analyses with 100% methylene chloride. The FID was a Tracor Model 945 operated as described previously (12). The detector signal was monitored by a real-time computer programmed to calculate peak area from solute responses (13). Quantitative response by FID was checked against weighed TAG standards. The HPLC effluent was passed through a 1:1 splitter, which diverted one-half of the HPLC stream to the detector and the remaining stream to an exit port for collection of TAG. Repeated injections of the TAG mixtures on the HPLC analytical column were performed to obtain 1–2 mg of individual TAG for characterization.

TAG characterization. Intact TAG isolated by RP-HPLC were characterized by ^1H and ^{13}C-nuclear magnetic resonance (NMR) in $CDCl_3$ as previously described (14–16). Further TAG characterization was by identification and determination of the quantity of TAG FA by capillary GC-FID of the respective methyl esters as described below.

Gas-liquid chromatography analysis. GC analysis of Ve and Cr TAG-FA after transmethylation was performed in triplicate on a 0.25 mm × 30 m Sp 2330 capillary column (Supelco Inc., Bellefonte, PA) operated at 170°C (10-min initial pause) to 220°C at 3°C/min. The area response of the GC FID detector and the identification of components were calibrated against standard mixtures of palmitic, stearic, vernolic and crepenynic FA methyl esters.

TABLE 2

Average Fatty Acid Compositions[a] at the Glycerol Moeity Carbons of the Triacylglycerols (TAG) in *Vernonia galamensis* and *Crepis alpina* Seed Oils

Fatty acid[b]	Fatty acid composition (area percent)			
	Glycerol carbons			
	Vernonia galamensis oil		*Crepis alpina* oil	
	2-	1,(3)-	2-	1,(3)-
P	0.1	4.6	0.7	5.8
S	0.7	3.3	0.3	2.1
O	6.9	2.9	1.3	3.7
L	26.0	9.5	6.1	28.0
Cr			91.6	60.4
Ve	65.6	79.9		

[a]Determined by TAG lipolysis to 2-monoglycerols followed by gas chromatographic analysis of the transmethylated monoglycerols, see the Experimental Procedures section.
[b]Cr, Crepenynic; Ve, vernolic; P, palmitic; S, stearic, O, oleic; L, linoleic.

RESULTS AND DISCUSSION

FA composition of the crude VeGO and CrAO used are given in Table 1. The oils were chromatographed by SE to remove non-TAG components before stereospecific and RP-HPLC-FID analyses. Non-TAG represented 1.3 and 5.0%, respectively, of the VeGO and CrAO chromatographed material. The non-TAG material was not characterized, but presumably contained tocopherols, sterols, pigments and other non-TAG components common to vegetable oils (12). The FA compositions of the TAG fractions of the chromatographed oils are in close agreement with the crude oils (Table 1), indicating that stereospecific and RP-HPLC-FID analyses of the chromatographed oils can be referenced to the TAG composition and structure of the crude oils.

Stereospecific analysis of VeGO (Table 2) show Ve to be more abundant on the 1,(3)-positions of the TAG. The com-

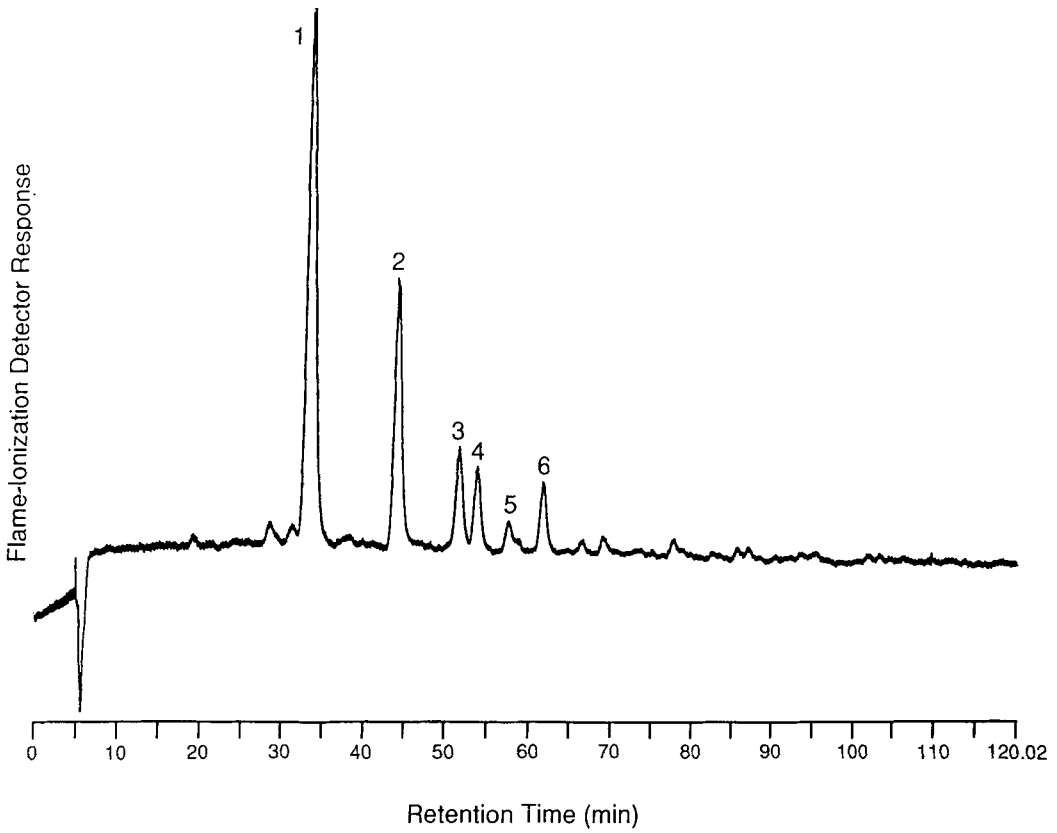

FIG. 1. Reversed-phase high-performance liquid chromatography (HPLC) analysis of chromatographed *Vernonia galamensis* seed oil. HPLC conditions: 0.5 mg sample, 5 μ C-18 column (0.49 × 50 cm); 120-min solvent gradient of acetonitrile/methylene chloride (70:30 to 40:60, vol/vol); flow rate, 0.5 mL/min; flame-ionization detector. Chromatogram peak numbers refer to triacylglycerol numbers in Table 7.

mon FAs, linoleic (L) and oleic (O), are located preferentially on the 2-position. The saturated FAs, stearic (S) and palmitic (P), are located on the 1,(3)-positions. The CrAO has Cr preferentially located on the 2-position. In contrast to common vegetable oils like soybean (SBO) (12), L is preferentially located on the 1,(3)-positions for CrAO. However, CrAO (like SBO) has saturated FA, P and S located on the 1,(3)-positions.

The chromatographed VeGO and CrAO TAGs were resolved by RP-HPLC-FID, as shown in Figures 1 and 2, respectively. The TAG identities were determined by proton NMR (^{1}H-NMR) for all TAG and by carbon-13 NMR (^{13}C-NMR) for the most abundant TAG of each oil and by capillary GC of the TAG after transmethylation.

The ^{1}H-NMR chemical shifts and proton assignments for the VeGO TAG fractions (Fig. 1) are presented in Table 3. HPLC fraction 1 was trivernoloyl glycerol (VeVeVe). This identification was based on the assignment of 6 protons (H) for 3 olefinic double bonds, 6 ring H for three epoxy units, 6 H for methylene units between olefinic and epoxy groups, 6 allylic H and 9 H for terminal methyl groups. The ^{1}H-NMR assignments for the H of the glycerol backbone were 1 H for carbon 2 and 4 H for carbons 1 and 3. These glycerol proton assignments were the same for all VeGO and CrAO TAG. The proton assignments for VeVeVe were supported by ^{13}C-NMR chemical shifts for carbons of HPLC fraction 1 as listed in Table 4. In addition to assignments for the glycerol carbons, assignments were present for vernolic acid for the epoxy carbon numbers 12 and 13, olefinic carbons 9 and 10, methylene carbon 14 (adjacent to epoxy carbon 13), methylene carbon 8 (adjacent to the *cis* 9,10 olefinic unit) and carbon 11 (between the epoxy and olefinic units). GC analysis of TAG HPLC fraction 1 after transmethylation showed the presence of 98% Ve and, with the NMR assignments, confirmed that HPLC fraction 1 was essentially VeVeVe.

HPLC fraction 2 was composed of divernoloyllinoleoyl glycerol (VeVeL). This identification was supported by the proton assignments previously discussed for VeVeVe, which indicated the presence of only two Ve acids in the TAG structure. The presence of one L in the TAG structure was supported by 2 H for the methylene group (between two olefinic groups), 8 H for methylene groups adjacent to two epoxy units of 2 Ve acids and allylic units adjacent to the diene structure on one L acid. The TAG structure was further supported by results of GC analysis, which showed 31.2% L and 64% Ve acid (see Table 5). The ^{1}H-NMR spectrum has been reported previously for methyl vernolate (17), but ^{1}H-NMR spectra have not been reported for trivernoloyl and divernoloyllinoleoyl glycerols. The ^{13}C-NMR spectrum (for methyl vernolate and trivernoloyl glycerol) have not been reported previously.

HPLC fractions 3–6 were identified as TAGs that contained 1 Ve and 2 FA each. ^{1}H-NMR results (Table 3)

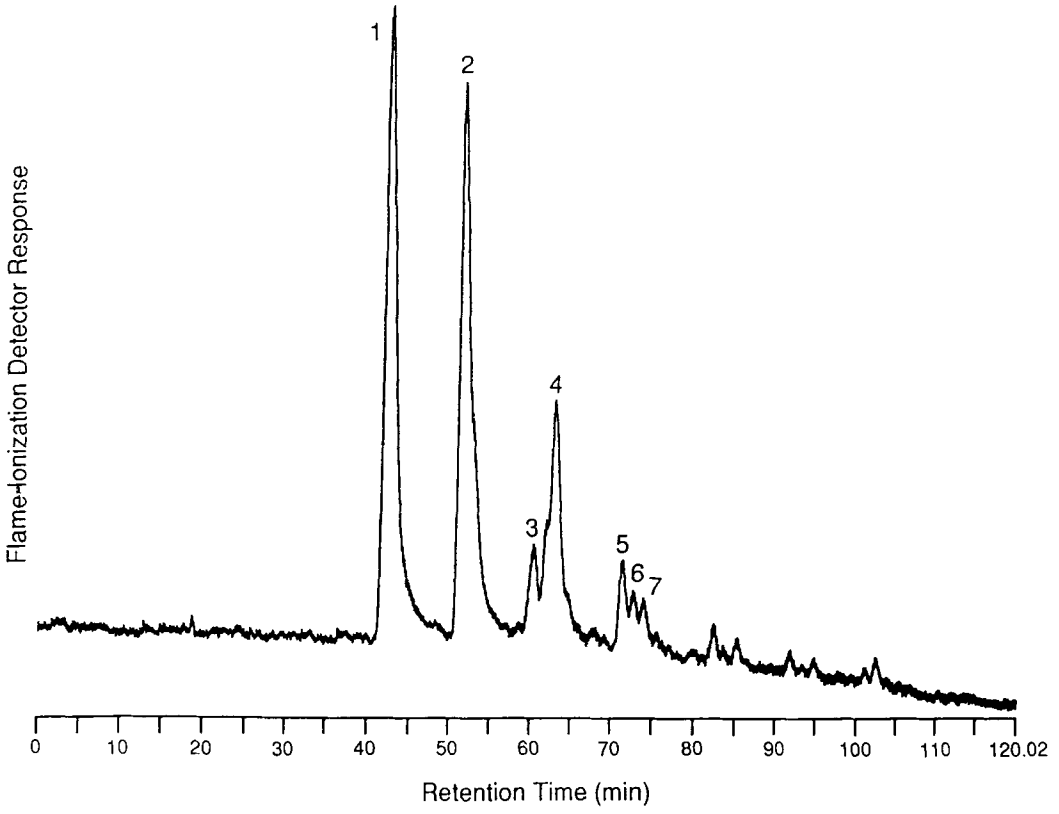

FIG. 2. Reversed-phase high-performance liquid chromatography (HPLC) of chromatographed *Crepis alpina* seed oil. HPLC conditions same as in Figure 1. Chromatogram peak numbers refer to triacylglycerol numbers in Table 7.

TABLE 3

^{1}H-Nuclear Magnetic Resonance ($CDCl_3$) of *Vernonia galamensis* High-Performance Liquid Chromatography Fractions[a]

TAG[b] fraction	ppm (multiplicity[c], number of protons, assignment[d])
1. VeVeVe	5.46 (*m*, 6, CH=CH), 5.25 (*m*, 1, COCHOCO), 4.21 (*m*, 4, CH_2OCOCH_2O), 2.95 (*t*, 6, CHOCH), 2.30 (*m*, 6, CH_2CO), 2.18 (*m*, 6, C = CCH_2COC), 2.01 (*m*, 6, CH_2C=C), 1.4-1.7 (*m*, CH_2CC=O, $COCCH_2$), 1.37 [*s*, $(CH_2)_n$], 0.95 (*t*, 9, CH_3)
2. VeVeL (VeLVe)	5.44 (*m*, 8, CH=CH), 5.26 (*m*, 1, COCHOCO), 4.21 (*m*, 4, CH_2OCOCH_2O), 2.92 (*m*, CHOCH), 2.76 (*m*, 2, C=CCH_2C=C), 2.35 (*m*, 6, CH_2CO), 2.18 (*m*, 4, C=CCH_2CHOCH), 2.05 (*m*, 8, CH_2C=C), 1.4-1.7 (*m*, CH_2CC=O, $COCCH_2$), 1.34 [*s*, $(CH_2)_n$], 0.95 (*m*, 9, CH_3)
3-6. VeFAFA	5.44 (*m*, CH=CH), 5.26 (*m*, 1, COCHOCO), 4.21 (*m*, 4, CH_2, $OCOCH_2O$), 2.92 (*m*, 2, CHOCH), 2.35 (*t*, 6, CH_2CO), 2.05 (*m*, 6, CH_2C=C), 1.4-1.7 (*m*, CH_2CC=O, $COCCH_2$), 1.35 [*s*, $(CH_2)_n$], 0.92 (*m*, 9, CH_3)

[a]See Figure 1.
[b]Ve, Vernolic; L, linoleic; TAG, triacylglycerol; FA, fatty acid.
[c]Multiplicity: *s* = singlet, *m* = multiplet, *t* = triplet.
[d]Proton assignments for vernolic acid of the tricylglycerol in $CDCl_3$ based on assignments reported for methyl vernolate in CCl_4 (17).

TABLE 4

^{13}C-NMR of Triacylglycerols (TAG) of 12,13-Epoxy-*cis*-9-octadecenoic acid (trivernoloyl glycerol, VeVeVe) and *cis* 9-Octadecen-12-ynoic acid (tricrepenynoyl glycerol, CrCrCr)

TAG[a,b]	Shifts, ppm (assignments)[c,d]
VeVeVe	173.2 (C=O), 132.5 (C=C$_{10}$), 124.0 (C$_9$=C), 68.9 (COCOCO),[e] 62.1 (COCOCO), 57.2 (C$_{12}$OC) 56.5 (COC$_{13}$), 34.2 (CC=O), 31.8 (C$_{16}$), 29.5, (C$_7$), 29.2 (C$_4$, C$_5$, C$_6$), 27.8 (C$_{14}$-epoxy), 27.4 (*cis* C$_8$C=C), 26.3 (C$_{11}$, C$_{15}$), 24.8 (C$_3$CC=O), 22.6 (C$_{17}$), 14.0 (C$_{18}$)
CrCrCr	173.2 (C=O), 132.5 (C$_9$=C), 125.1 (C=C$_{10}$), 80.1 (C≡C$_{13}$), 78.3 (C$_{12}$≡C), 68.9 (COCOCO),[e] 6.21 (COCOCO), 34.2 (C$_2$C=O), 31.1 (C$_{16}$CCH$_3$, 29.3 (C$_7$), 29.1 (C$_4$,C$_5$,C$_6$), 28.8 (C$_{15}$), 27.1 (*cis* C$_8$C=C), 24.8 (C$_3$), 22.2 (C$_{17}$), 18.8 (C≡CC$_{14}$), 17.2 (C=CC$_{11}$C≡C), 14.0 (CH$_3$)

[a]See Figures 1 and 2.
[b]Ve, Vernolic; Cr, crepenynic.
[c]Assignments for VeVeVe not previously reported but are based on similar epoxy compounds.
[d]Assignments for CrCrCr in $CDCl_3$ based on methyl crepenynate (Ref. 19).
[e]TAG glycerol carbons.

TABLE 5

Fatty Acid Compositions of the Triacylglycerols[a,b]

	GC flame ionization area percent[c] HPLC fraction												
	Vernonia galamensis						*Crepis alpina*						
Acid[d]	1	2	3	4	5	6	1	2	3	4	5	6	7
P	0.2	2.2	9.9	44.2	14.5	14.5	1.4	0.5	14.2	16.9	8.6	22.8	17.8
S		0.8	3.9	3.7	5.5	5.5	0.3		6.9		21.4	20.4	25.4
O		1.7	50.2	13.3	15.7	15.7			10.9	18.4	18.8	13.8	14.3
L	1.7	31.2	6.6	5.3	30.6	37.6		30.2	5.3		19.6	13.3	13.8
Ve	98.0	64.1	29.4	33.5	33.7	26.8							
Cr							98.3	69.3	62.7	64.7	31.6	29.7	28.7
Ve/FA[e]		1.8	0.4	0.5	0.5	0.4							
Cr/FA[f]								2.3	2.2	1.8	0.5	0.5	0.4

[a]See Figures 1 and 2.
[b]High-performance liquid chromatography (HPLC) was performed on the pure triacylglycerol fractions; see Experimental Procedures section for details.
[c]Gas chromatography (GC) performed on the transmethylated HPLC fractions; see Experimental Procedures section for details.
[d]Abbreviations as in Table 2.
[e]Ratio of methyl vernolate to other fatty acids (FA).
[f]Ratio of methyl crepenynate to other FA.

show that each fraction, 3–6, contained 2 H for the epoxy unit, consistent with only one Ve acid per TAG. GC analysis of fractions 3–6 after transmethylation showed Ve/FA ratios of 0.4–0.5 (Table 5), which was consistent with two FA and 1 Ve per TAG.

The ^{1}H-NMR chemical shifts and proton assignments for the CrAO TAG fractions (Fig. 2) are presented in Table 6. HPLC fraction 1 was composed of tricrepenynoyl glycerol (CrCrCr). This identification was supported by the assignment of 6 H for methylene between olefinic and acetylenic and 6 H for methylene adjacent to acetylenic units. Other assignments were similar to those for VeVeVe except for the absence of epoxy-related protons. The proton assignments for CrCrCr were supported by ^{13}C-NMR for those carbons of HPLC fraction 1 listed in Table 4. In addition to assignments for the glycerol 2- and 1,(3)-carbons, assignments were made for the acetylenic carbons 12 and 13, olefinic carbons 9 and 10, methylene carbon 14 (adjacent to acetylenic carbon 13), methylene carbon 8 (adjacent to the 9,10 olefinic unit) and carbon 11 (between the olefinic and acetylenic units). GC analysis of the HPLC fraction 1 after transmethylation showed the presence of 98% Cr, which supported the NMR assignments and confirmed that HPLC fraction 1 was essentially CrCrCr (Table 5).

HPLC fraction 2 was composed of dicrepenynoyllinoleoyl glycerol (CrCrL). This identification was supported by the proton assignments previously discussed for CrCrCr, which indicated the presence of two Cr acids in the TAG structure. The presence of one L in the TAG

TABLE 6

^{1}H-Nuclear Magnetic Resonance of *Crepis alpina* High-Performance Liquid Chromatography Fractions[a]

TAG[b] fraction	ppm (multiplicity[c], number of protons, assignment[d])
1. CrCrCr	5.40 (*m*, 6, CH=CH), 5.25 (*m*, 1, COCHOCO), 4.21 (*m*, 4, CH_2OCOCH_2O), 2.89 (*m*, 6, $C=CCH_2C\equiv C$), 2.32 (*t*, 6, $CH_2C=O$), 2.14 (*m*, 6, $CH_2C\equiv C$), 2.05 (*m*, 6, $CH_2C=C$), 1.64 (*m*, 6, $CH_2CC=O$), 1.30 [*m*, $(CH_2)_n$], 0.91 (*t*, 9, CH_3)
2. CrCrL (CrLCr)	5.39 (*m*, 8, CH=CH), 5.25 (*m*, 1, COCHOCO), 4.21 (*m*, 4, CH_2OCOCH_2O), 2.89 (*m*, 4, $C=CCH_2C\equiv C$), 2.75 (*m*, 2, $C=CCH_2C=C$), 2.30 (*t*, 6, CH_2CO), 2.12 (*m*, 4, $CH_2C\equiv C$), 2.05 (*m*, 8, $CH_2C=O$), 1.64 (*m*, 6, CH_2CCO), 1.30 [*m*, $(CH_2)_n$], 0.90 (*t*, 9, CH_3)
3-4. CrCrFA	5.38 (*m*, 6, CH=CH), 5.25 (*m*, 1, COCHOCO), 4.21 (*m*, 4, CH_2OCOCH_2O), 2.89 (*m*, 4, $C=CCH_2C\equiv C$), 2.32 (*t*, 6, CH_2CO), 2.12 (*m*, 4, $CH_2C\equiv C$), 2.05 (*m*, 8, $CH_2C=C$), 1.64 (*m*, 6, $CH_2CC=O$), 1.30 [*m*, $(CH_2)_n$] 0.90 (*m*, 9, CH_3)
5-7. CrFAFA	5.38 (*m*, CH=CH), 5.25 (*m*, 1, COCHOCO), 4.21 (*m*, 4, CH_2OCOCH_2O), 2.89 (*m*, 2, $C=CCH_2C\equiv C$), 2.78 (*m*, $C=CCH_2C=C$), 2.32 (*t*, 6, CH_2CO), 2.12 (*m*, 2, $CH_2C\equiv C$), 2.05 (*m*, $CH_2C=C$), 1.64 (*m*, CH_2CCO), 1.30 [*m*, $(CH_2)_n$], 0.90 (*m*, 9, CH_3)

[a]See Figure 2 for RP-HPLC-FID chromatogram of *Crepis alpina* triacylglycerols (TAG) fractions 1-7.
[b]Cr, Crepenynic; L, linoleic; FA: palmitic, stearic, oleic and linoleic acids.
[c]Multiplicity: *s* = singlet, *m* = multiplet, *t* = triplet.
[d]Proton assignments for crepenynic acid of the TAG in $CDCl_3$ based on assignments reported for methyl crepenynate in CCl_4 (Ref. 18).

structure was supported by the same ^{1}H-NMR assignments presented for L in VeVeL. The TAG structure was further supported by results of GC analysis, which showed 30.2% L and 69.3% Cr (Table 5). The ^{1}H-NMR (18) and ^{13}C-NMR spectra have been reported previously (19) for methyl crepenynate. However, the ^{1}H-NMR data have not been reported previously for tricrepenynoyl- and dicrepenynoyllinoleoyl glycerols. The ^{13}C-NMR data for tricrepenynoyl glycerol have not been reported before.

Results of ^{1}H-NMR (Table 6) and GC (Table 5) supported the designation of HPLC fractions 3 and 4 (Fig. 2) as TAGs which contained 2 Cr and 1 FA each. ^{1}H-NMR results (Table 6) showed that each fraction contained 2 Cr groups. The GC (Table 5) analysis indicated that HPLC fraction 3 contained TAGs with 2 Cr and FA, which included L, O, S or P, and that fraction 4 contained TAG with 2 Cr and either 1 P or 1 S.

HPLC fractions 5-7 (Fig. 2) were identified as TAG composed of 1 Cr and 2 FA each. Results of ^{1}H-NMR (Table 6) showed that each fraction contained 1 Cr group. GC analysis (Table 5) indicated that fractions 5-7 contained TAGs with 1 Cr and 2 FA, which include L, O, S and P.

Quantitative TAG analyses of VeGO and CrAO obtained in triplicate by RP-HPLC-FID (Figs. 1 and 2) are presented in Table 7. TAG standards containing saturated FA with chainlengths of 6-18 carbons, by weight, were used to quantitate the FID-HPLC detector response. In the area of interest for VeGO and CrAO (HPLC retention 20-80 min), HPLC-FID area percent was within 0.1-1.7% of the wt% of the TAG standard components. Therefore, the TAG composition as area percent by RP-HPLC-FID for VeGO and CrAO is likely within 2% of TAG wt%. In addition, quantitative linear response of the FID-HPLC detector was previously verified for SBO TAG (20).

For VeGO and CrAO TAG identification by RP-HPLC-FID retention times, retention data of each TAG is reported in Table 7 in absolute time and also in reference to the time of retention of standard TAG with saturated FA of total carbon numbers C18, C39 and C42. Carlson and Chang (4) performed high-temperature capillary GC on the TAG of a VeGO and found 59% VeVeVe, 28% VeVeFA and 9.5% VeFAFA. These investigators determined by quantitative column chromatography that the VeGO contained only 48% VeVeVe, which compares closely with our value of 50% from RP-HPLC analysis (Table 7). Their GC results may differ from those of RP-HPLC-FID due to need for response factors for the GC data. The Ve content in several varieties has been reported to be 70-80% (1,2), indicative of the existence of VeGO varieties with different TAG compositions. This is reasonable because we have previously found considerable differences in TAG composition of oils from different soybean varieties (12).

TABLE 7

Reversed-Phase High-Performance Liquid Chromatography with Flame-Ionization Detection Analysis of the Triacylglycerols (TAG) of *Vernonia galamensis* and *Crepis alpina* Seed Oils[a]

Oil seed	TAG[b]	Triacylglycerol retention time				Triacylglycerol quantitative analysis area percent
		Absolute time (min)	RRT[c]			
			CaCaCa	LaLaLa	MyMyMy	
Vernonia galamensis	1. VeVeVe	33.00 ± 0.5	0.92	0.57	0.39	50.3 ± 0.8
	2. VeVeL	43.77 ± 0.48	1.22	0.75	0.52	20.6 ± 0.3
	3. VeFAFA	51.51 ± 0.58	1.43	0.89	0.61	7.4 ± 0.8
	4. VeFAFA	53.70 ± 0.57	1.49	0.92	0.63	5.6 ± 0.5
	5. VeFAFA	57.48 ± 0.53	1.60	0.99	0.68	1.8 ± 0.2
	6. VeFAFA	61.61 ± 0.53	1.71	1.06	0.73	4.7 ± 0.2
Crepis alpina	1. CrCrCr	42.28 ± 0.33	1.17	0.73	0.50	36.4 ± 1.4
	2. CrCrL	51.16 ± 0.71	1.42	0.88	0.60	33.4 ± 0.9
	3. CrCrFA	59.71 ± 0.85	1.66	1.03	0.70	3.4 ± 0.4
	4. CrCrFA	62.30 ± 0.85	1.78	1.07	0.74	13.6 ± 0.3
	5. CrFAFA	70.86 ± 0.77	1.97	1.22	0.84	3.3 ± 0.1
	6. CrFAFA	72.15 ± 0.68	2.00	1.24	0.85	1.6 ± 0.5
	7. CrFAFA	73.40 ± 0.59	2.04	1.26	0.87	1.6 ± 0.2

[a]See Figures 1 and 2.
[b]Ve, Vernolic; Cr, crepenynic; L, linoleic; FA: palmitic, stearic, oleic and linoleic acids.
[c]RRT, relative retention times of Ve and Cr TAG with respect to TAG of these fatty acids: Ca, capric; La, lauric; My, myristic.

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