Mass Spectrometric Characterization of *Vernonia galamensis* Oil

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Vernonia galamensis **seed oil is a natural source of epoxidized triacylglycerols, which consist of 52% trivernolin and a mixture of other triacylglycerols. Epoxidized oils are used for industrial applications, such as coatings and plastic formulations. To determine the major molecular species present in Vernonia oil, desorption chemical ionization/mass spectrometry and mass spectrometry/mass spectrometry were used to determine its glyceride composition. Seven triacylglycerols predominated: divernoloylarachidonate, trivernolin, divernoloylstearate, divernoloyloleate, divernoloyllinoleate, dilinolenoyl vernolate and divernoloylpalmitate.**

KEY WORDS: Desorption chemical ionization, mass spectrometry, triacylglycerols, vernolic acid, *Vernonia galamensis*, Vernonia oil.

Vernonia galamensis oil (VO) is a naturally epoxidized seed oil, the triacylglycerol composition of which has been characterized by thin-layer chromatography and gas chromatography (1). These techniques demonstrated that VO is largely composed of triacylglycerols that contain vernolic (*cis*-12,13-epoxy-*cis*-9,10-octadecenoic) acid, of which 59.2% is trivernolin, 28.1% is divernoloyl triglycerides, 9.5% monovernoloyl triglycerides and 3.3% is nonvernoloyl triglycerides (1).

As a result of continued interest in VO for both industrial and synthetic applications, we have developed mass spectrometric techniques to characterize VO with respect to its molecular distribution. Initially, in order to calculate the theoretical distribution of the component triacylglycerols, assuming that they were randomly distributed, we used the following literature values (1–3) for the fatty acid composition of VO: Vernolic acid (VER), 75%; linoleic acid (LIN), 13%; oleic acid (OLE), 6%; stearic acid (STE), 3%; palmitic acid (PAL), 2.5%; and arachidic acid (ARA), 0.5%. To determine this distribution, we had to satisfy the conditions of a system consisting of six fatty acids that are independently taken three at a time. Subsequently, the probability of each molecular species was calculated with the molecular formula:

$$P = m \cdot (X_{j1} \cdot X_{j2} \cdot X_{j3}) \qquad [1]$$

where m represents the number of possible arrangements of each molecular species (for example, VER-OLE-LIN has six possible arrangements while VER-VER-VER has only one) and X_{ji} represents the mole fraction of each of the six fatty acids. This results in $[6]^3$ or 216 possible arrangements. The number of possibilities was reduced by the fact that, for instance, VER-OLE-LIN has the same mass as VER-LIN-OLE. This consideration leads to 56 distinct combinations. Twenty-five of these combinations have unique molecular weights. The other 31 are distributed among 14 different molecular weights. Further scrutiny of these combinations, in which we focused on those triacylglycerols with greater than 0.01 probability, resulted in 11 triacylglycerol species (Table 1). This was further reduced to 10, considering that mass 926 was attributable to VER-VER-VER and VER-LIN-ARA.

Thus, the present study focused on the determination of the molecular distribution of VO. The experiments included desorption chemical ionization/mass spectrometry (DCI/MS) and DCI/MS/MS.

EXPERIMENTAL PROCEDURES

A triple quadrupole mass spectrometer with unit resolution at 1000 amu (Finnigan MAT TSQ 46, San Jose, CA) (mass range in the first quadrupole was 10–1900 amu and 10–1800 amu in the third quadrupole) was used to determine the molecular distribution of VO. DCI (with methane and isobutane as reagent gases) in positive-ionization mode was used. In DCI (a soft-ionization procedure), the samples were deposited onto the DCI filament and placed into the ion source (Fig. 1). To volatilize the sample, a current from 0–1300 mA was sent through the DCI filament at rates of 0.5–1000 mA per second.

The triple quadrupole mass spectrometer was capable of performing MS/MS. In this experiment, the first quadrupole selected the "parent" ion (in this case, the molecular ion). Then, the second quadrupole fragmented the parent ion with a collision gas to produce "daughter" ions, and the third quadrupole analyzed the daughter ions that were sent to the detector.

DCI (methane, 0.4 torr) was used to obtain qualitative triglyceride information. Samples of VO (100 ng/μL in hexane) were volatilized in the range of 0–400 mA at 20 mA/s. The samples were scanned (0.50 scans/s) over a range of 250–1000 amu with electron multiplier voltage of 1000 V.

MS/MS experiments were performed on VO with methane (0.4 torr) as the reagent gas and xenon (0.01 mtorr) as the collision gas. The same range of 0–400 mA at 20 mA/s was used for MS/MS on masses 928, 910, 912, 914, 916, 944, 888 and 896.

The crude VO used in this study was obtained from mechanical pressing of enzyme-deactivated *Vernonia*

TABLE 1

Major Triglyceride Components of *Vernonia galamensis* Oil Based on Theoretical Calculation

Combinations[a]	Probabilities	Theoretical percentages
VER-VER-VER	.4219	44.3
VER-VER-LIN	.2194	23.1
VER-VER-OLE	.1013	8.7
VER-VER-STE	.0506	5.3
VER-VER-PAL	.0422	4.4
LIN-LIN-VER	.0380	4.0
VER-LIN-OLE	.0351	3.7
VER-LIN-STE	.0176	2.0
VER-LIN-PAL	.0146	2.0
VER-VER-ARA	.0084	0.9
VER-LIN-ARA	.0029	0.3

[a]VER, vermolic acid; LIN, linoleic acid; OLE, oleic acid; STE, stearic acid; PAL, palmitic acid; ARA, arachidic acid.

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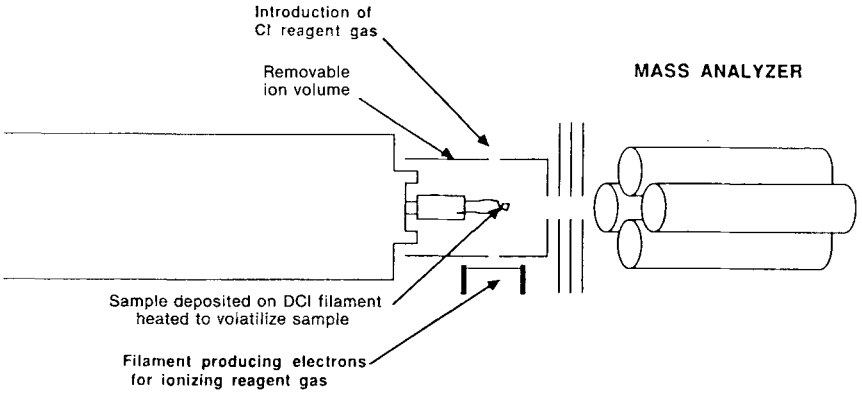

FIG. 1. Direct analysis of Vernonia oil by desorption chemical ionization (DCI)/mass spectrometry.

galamensis seed (4). Titrimetric analysis (5) gave a weight per epoxy of 415, which translates to an oxirane percentage of 3.86 (74.5% vernolic acid).

RESULTS AND DISCUSSION

Seven major triacylglycerols were identified in VO by using DCI/MS/MS (Table 2). A current of 0–400 mA at 20 mA/s provided the optimum abundance of parent ions (Fig. 2). This spectrum has three distinct regions: fragment ions from 200 to 350 amu; diglyceride fragment ions from 600 to 650 amu; and triacylglycerol ions from 800 to 950 amu. The triacylglycerol region was examined in greater detail by replotting over the mass range of 780–980 amu (Fig. 3). From this region, we used the even-numbered ions to identify the triacylglycerols based on our calculated molecular species distribution. Of the 56 possible species, only seven, most of them containing palmitic acid moieties, were not present. This was expected, considering the fact that the seven species are among those with the lowest probability factors. Thus, parent ions corresponding to 49 triacylglycerol species were identified. However, because of the uncertainty of the contribution due to isotopic distribution and also because of the MS background noise, further analysis by MS/MS was performed on only seven major ions. These seven species (Table 2) were present in our random distribution. In addition, there was a prominent ion at *m/z* 910, which does not correspond to any triacylglycerol in the random distribution, the best fit of which was VER-VER-LINO (linolenic acid). However, as LINO is not

TABLE 2

Seven Major Triglycerides Identified from Desorption Chemical Ionization/Mass Spectrometry of Vernonia Oil

Triglyceride[a]	Nominal mass	Exact mass	Observed[b]
VER-VER-ARA	942	942.79	944
VER-VER-VER	926	926.72	928
VER-VER-STE	914	914.76	916
VER-VER-OLE	912	912.74	914
VER-VER-LIN	910	910.73	912
VER-LIN-LIN	894	894.73	896
VER-VER-PAL	886	886.73	888

[a]Abbreviations as in Table 1.
[b]Observed equals the exact mass +1 (rounded off).

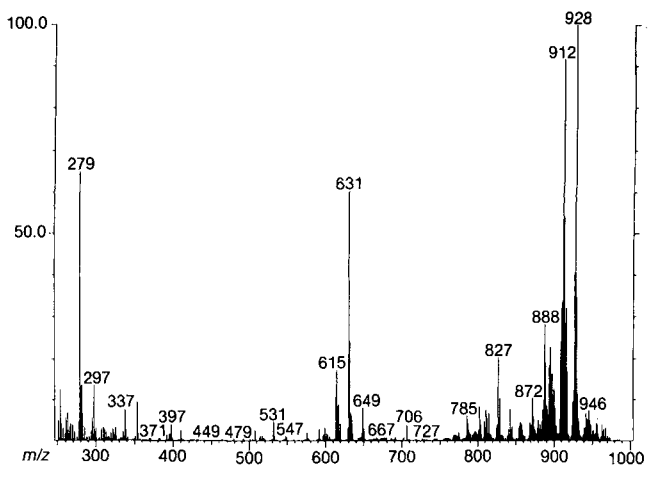

FIG. 2. Desorption chemical ionization/mass spectrometry/mass spectrometry of Vernonia oil from 200 to 1000 amu.

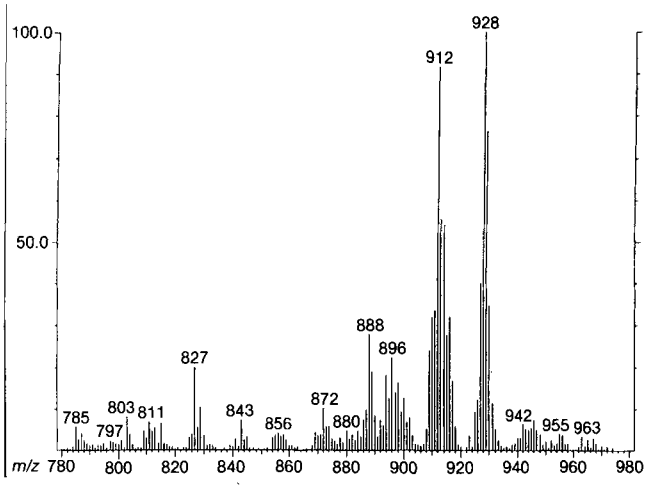

FIG. 3. Desorption chemical ionization/mass spectrometry/mass spectrometry of Vernonia oil, showing triglyceride region from 780 to 980 amu.

indicated in the methyl ester analysis of VO, we concluded that the ion at *m/z* 910 (corresponding to *m/z* 908) was due to the loss of water from trivernolin. This conclusion was supported by the fact that the MS/MS analysis of the trivernolin ion at *m/z* 928 (Figs. 4) gave two diglyceride moieties at *m/z* 631 (VER-VER) and *m/z* 613 (VER-C18:3).

DCI/MS/MS was performed to obtain structural information on the seven major triacylglycerols (Figs. 4–9). The MS/MS spectrum of VER-VER-ARA is not provided because of the relatively high background noise due to the low intensity of the parent ion at *m/z* 943.81. The parent ions were examined in a probe current range of 0–400 mA at a rate of 20 mA/s. The ions of interest were the diglyceride fragment ions (Table 3), which confirmed the molecular distributions but not the positions of the fatty acids on the glycerol moiety. All the triacylglycerols had two or more vernolic acids, except for one which had one vernolic acid and two linoleic acids. This is not surprising because these two fatty acids are the major acids in

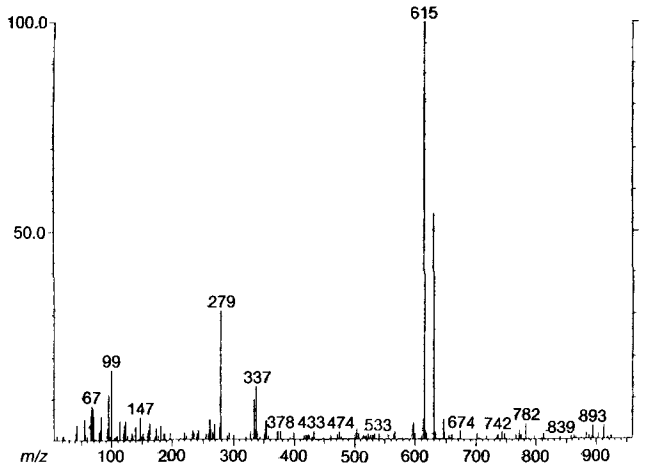

FIG. 5. Desorption chemical ionization/mass spectrometry/mass spectrometry of *m/z* 912 corresponding to VER-VER-LIN (mw 910); *m/z* 631 = VER-VER, *m/z* 615 = VER-LIN. Abbreviations as in Table 1.

TABLE 3

Desorption Chemical Ionization/Mass Spectrometry/Mass Spectrometry of Vernonia Oil[a]

Parent ion	Lost fragment	Daughter ion	Daughter ion species
927.81	VER	631	VER-VER
	VER + H_2O	613	VER-C18:3
911.81	LIN	631	VER-VER
	VER	615	VER-LIN
913.87	OLE	631	VER-VER
	VER	617	VER-OLE
915.87	STE	631	VER-VER
	VER	619	VER-STE
943.81	VER	648	VER-ARA
	ARA	631	VER-VER
887.81	PAL	631	VER-VER
	VER	591	VER-PAL
895.81	LIN	615	VER-LIN
	VER	599	LIN-LIN

[a]Abbreviations as in Table 1.

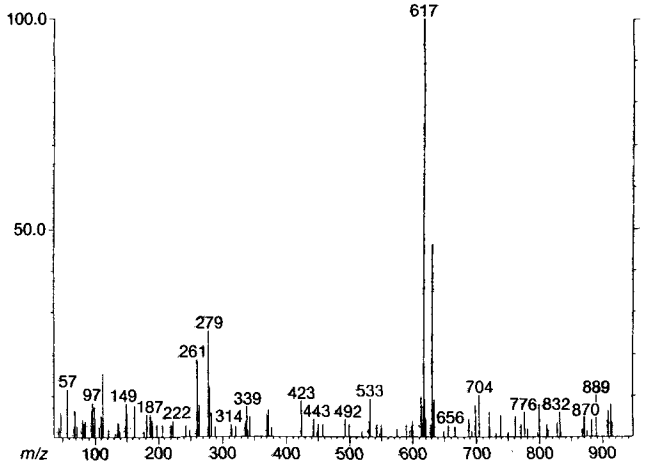

FIG. 6. Desorption chemical ionization/mass spectrometry/mass spectrometry of *m/z* 914 corresponding to VER-VER-OLE (mw 912); *m/z* 631 = VER-VER, *m/z* 617 = VER-OLE. Abbreviations as in Table 1.

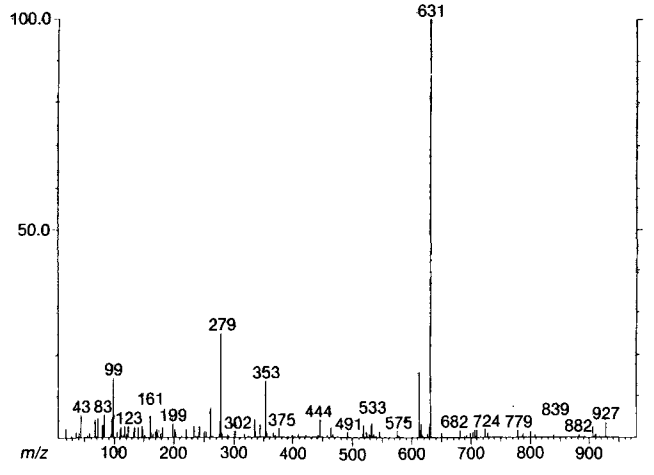

FIG. 4. Desorption chemical ionization/mass spectrometry/mass spectrometry of *m/z* 928 corresponding to VER-VER-VER (mw 926); *m/z* 631 = VER-VER, *m/z* 613 = VER-C18:3. Abbreviations as in Table 1.

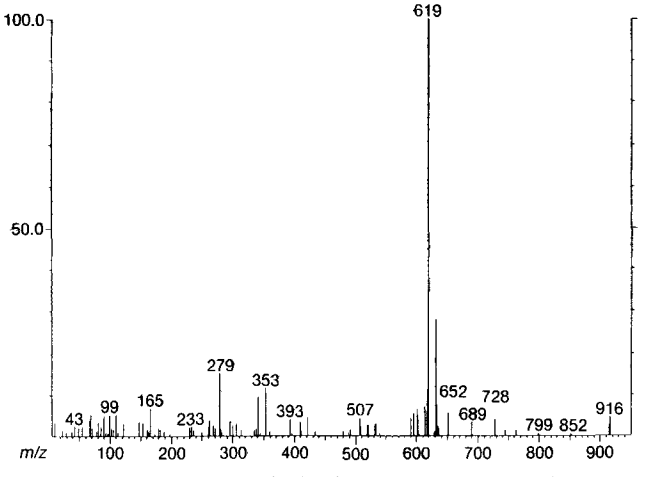

FIG. 7. Desorption chemical ionization/mass spectrometry/mass spectrometry of *m/z* 916 corresponding to VER-VER-STE (mw 914); *m/z* 631 = VER-VER, *m/z* 619 = VER-STE. Abbreviations as in Table 1.

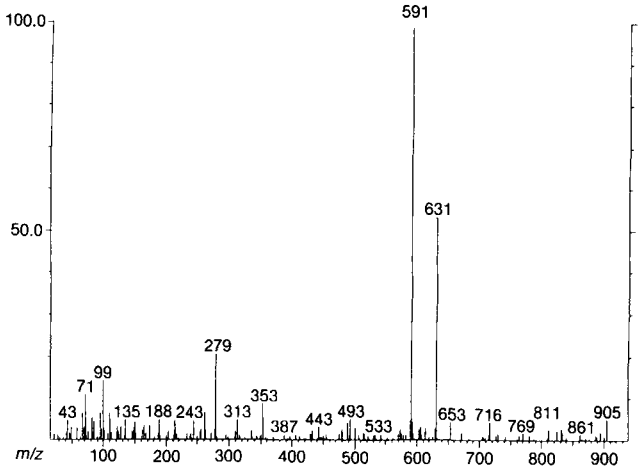

FIG. 8. Desorption chemical ionization/mass spectrometry/mass spectrometry of *m/z* 896 corresponding to VER-LIN-LIN (mw 894); *m/z* 615 = VER-LIN, *m/z* 599 = LIN-LIN. Abbreviations as in Table 1.

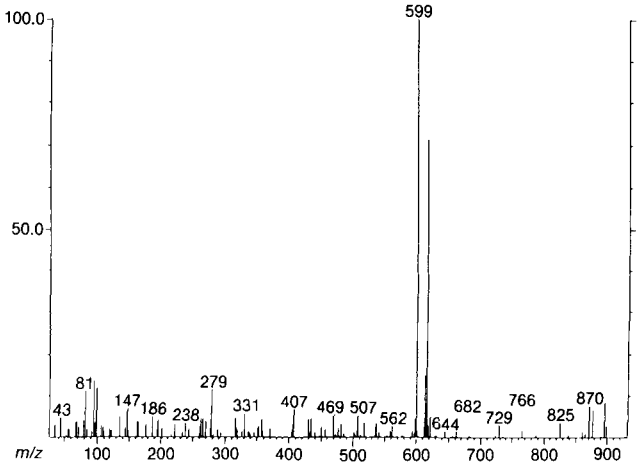

FIG. 9. Desorption chemical ionization/mass spectrometry/mass spectrometry of *m/z* 888 corresponding to VER-VER-PAL (mw 886); *m/z* 631 = VER-VER, *m/z* 591 = VER-PAL. Abbreviations as in Table 1.

Vernonia oil, and are in agreement with our calculated triacylglycerol random distribution. Furthermore, this finding is consistent with the earlier studies by Carlson and Chang (1), in which more than 85% of VO triacylglycerols were reported to consist of two or three vernolic acid moieties.

The triacylglycerol structures were confirmed by MS/MS, which we focused on the corresponding diglyceride ions (Table 3). However, in an attempt to quantitate the molecular species based on the ion intensities, it became apparent that the presence of vernolic acid on a glyceride molecule increased the tendency for fragmentation. For example (Figs. 5–7), the diglyceride fragments at *m/z* 615 and 631 (VER-LIN and VER-VER) gave a 2:1 ratio, whereas the ratio of *m/z* 617 and 631 (VER-OLE and VER-VER) was about 7:3, and *m/z* 619 and 631 (VER-STE and VER-VER) was about 3:1. Thus, the lower tendencies for fragmentation by the nonepoxy acids probably was responsible for the unexpectedly high intensities of the VER-VER-LIN ion at *m/z* 912 relative to VER-VER-VER at *m/z* 928 (Fig. 2). Consequently, for mass spectrometry to be a useful tool for quantitation of the triacylglycerols in VO, reference standards that contain the corresponding molecular species must be used. However, DC/MS/MS provided excellent qualitative data with respect to the identification of the major triacylglycerols in VO.

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