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ORIGINAL PAPER

Vernonia Oil: Conversion to a Mixture of Tertiary Amines Including *N*,*N*-Dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenyl Amine

Nikki S. Johnson · Folahan O. Ayorinde

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Abstract Vernonia galamensis is a new potential industrial oilseed crop found in tropical Africa. It is the source of a naturally epoxidized oil called vernonia oil (VO) which is extracted from the seed of the plant. In this study VO was used as the starting material for the synthesis of a mixture of amines, with the major product amine being *N*,*N*-dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenyl amine. VO was transesterified via a base catalyzed methanolysis using sodium methoxide to yield VO methyl esters (VOME). Aminolysis of the VOME with dimethylamine as reagent and solvent under reflux conditions afforded the tertiary amides, with *N*,*N*- dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenyl amide as the major product. The mixture was then subjected to metal hydride reduction with lithium aluminum hydride in diethylether under reflux conditions to obtain the desired amine mixture including *N*,*N*-dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenyl amine. Electron impact mass spectrometry was used to characterize the mixture of amines. Additionally, proton NMR, ^{13}C NMR, and FTIR were used for characterization of *N*,*N*-dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenyl amine. To further confirm the conversion of VO to the amines, the quaternary ammonium salts were synthesized and characterized by matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

Keywords Vernonia oil · Oilseed · Epoxy amide · Epoxy amine · MALDI-TOF

Introduction

Vernonia oil is a naturally epoxidized oil that is extracted from the seeds (40% oil) of the *Vernonia galamensis* plant which is a biorenewable resource that grows wild as weeds in tropical Africa [1]. The oil is a complex mixture of triacylglycerols, primarily made up of trivernolin, with the predominant acid moiety being (+)-(12*S*,13*R*)-epoxy-*cis*-9-octadecenoic (vernolic) acid. Upon hydrolysis, VO triacylglycerols yield about 72–80% vernolic acid [2, 3]. It is the epoxy functionality in vernonia oil that makes it unique in comparison to other vegetable oils such as coconut oil, palm kernel oil, soybean oil, sunflower oil, etc., of which none contain the epoxy functionality in the same percentage found in vernonia oil. Epoxidized fatty acids and their derivatives are of great value to industry because they are used in plastic formulations and polymer blends/coatings [4]. Currently the production of epoxidized fatty acids and derivatives is from expensive chemical epoxidation of vegetable oils such as soybean oil and linseed oil [5, 6].

Fatty amines are important raw materials in the oleochemical industry, and they are intermediates in the production of cationic surfactants [7, 8]. Amines have been synthesized from the amination of alcohols [9] and of alkyl halides [10] as well as from the reduction of amides [11] and of nitriles [12]. Further, functionalized amines such as epoxy fatty amines are useful intermediates in the production of industrial products such as polymers [13, 14]. Epoxidized oleochemicals have been successfully utilized as lubricants, antirust agents, and resins [15–17]. Because vernonia oil and its derivatives naturally contain both epoxy and double bond functionalities, oleochemicals derived from the oil would be of great value to industry.

Here we report a facile synthesis of a tertiary amine mixture from vernonia oil, with the major product being

N. S. Johnson · F. O. Ayorinde (✉)
Department of Chemistry, Howard University,
525 College St. NW, Washington, DC 20059, USA
e-mail: fayorinde@howard.edu

N,*N*-dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenyl amine. Amines from this oil are potential intermediates in the production of unique surfactants. For characterization purposes we also report the synthesis of a mixture of quaternary ammonium salts with the predominant salt being *N*,*N*,*N*-ethyl dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenyl ammonium bromide derived from the aforementioned tertiary amine mixture.

Experimental Procedures

Reagents

Crude VO was acquired from International Exchange of Trade and Technology Inc. (Culver, IN, USA). Sodium methoxide in methanol, hexane, dimethylamine, diethyl ether, lithium aluminum hydride, sodium sulfate, ethanol, and, bromoethane were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA).

Instrumentation

Monitoring of reactions was with an Agilent 6890N gas chromatograph interfaced with an Agilent 5973 inert mass spectrometer. The interface oven was maintained at 250 °C, the ionizer temperature setting was at 230 °C, using electron ionization (EI) with electron energy at 70 eV. High resolution capillary gas chromatography was conducted with a Supelco fused-silica SPB-5 (15 m, 0.25 mm ID, 0.25 μm film) column (Bellefonte, PA, USA), oven temperature was programmed from 50 to 300 °C (20 °C/min), and helium was used as carrier gas with head pressure 9.8 psi. IR spectra were collected on a Perkin Elmer Spectrum 100 FTIR spectrometer. The ^{13}C nuclear magnetic resonance (^{13}C NMR), proton nuclear magnetic resonance (^{1}H NMR), and DEPT-135 spectra were recorded on a Bruker Avance 400 MHz spectrometer with either chloroform-d ($CDCl_3$) or deuterium oxide (D_2O) as solvent. For characterization purposes the quaternary ammonium salts of the amine mixture were synthesized. The molecular masses were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) using an Applied Biosystems Voyager-DE STR BioSpectrometry Workstation equipped with a two-stage acceleration ion source. Positive ion MALDI spectra (200 summed acquisitions) were acquired in delayed-extraction (150 ns) and reflector modes. The acceleration voltage was 20 kV, the grid voltage at 75%, nitrogen laser (337 nm, 3 ns pulse width), and low mass gate at *m*/*z* 50. The matrix, meso-tetrakis(pentafluorophenyl)porphyrin (F20TPP), was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Matrix solution (10 mg/mL $CHCl_3$) and analyte solution {2 mg/mL H_2O} were mixed (70/30 μL) in 2-mL Eppendorf microcentrifuge tubes. After vortexing for 10 s, 1 μL of the sample solution was deposited on the sample plate and then allowed to evaporate at room temperature to enable co-crystallization of matrix and analyte.

Transesterification of VO to VOME (1)

A previously reported procedure by Elhilo et al. [18] was followed without modification for the conversion to the methyl esters.

Amidation of VOME

To a 50-mL round-bottom flask equipped with a magnetic stir bar, was added VOME (**1**) (3.12 g, 10.01 mmol), followed by 10 mL of dimethylamine (1 M solution in methanol), and 2.4 mL of sodium methoxide (25 wt%). After 2 h of reflux, the reaction mix was quickly transferred to a 50-mL beaker and placed in an ice bath (0 °C) for 15 min and then allowed to solidify for 24 h. Next, the solidified product was carefully washed with 5-mL ethanol. A pale yellow solid (1.11 g, 34.23%) resulted. GC–MS data revealed a mixture of palmitamide, oleamide, stearamide, and the major product vernolamide (**2**). The pseudo-molecular ion for vernolamide (**2**) was at *m*/*z* 324 [M + H] with diagnostic ions at *m*/*z* 87, 210 [M-113], and 252 [M-71]. IR: 1,631.21 and 1,552.52 cm^{-1}, 842.13 and 821.71 cm^{-1}. ^{1}H: (MeOD) δ 0.84 (t, 3H, CH_3), 1.18–1.97 (m, 20H, 10 CH_2), 2.17 (t, 2H, CH_2C=O), 5.40–5.60 (m, 2H, CH=CH), 2.4 (m, 2H, CH_2–CHOCH), 2.95 (broad, 2H, epoxy, CHCH), 3.21 (s,6H, N(CH_3)$_2$); ^{13}C: (D_2O) δ 13.9 (1C, CH_3), 22.0–36.0 (10C, CH_2), 37.4 (1C, CH2C=O), 37.8 (1C, CH2CHOCH), 55.8 (2C, N(CH$_3$)$_2$), 56.6 and 57.3 (2C, epoxy CHCH), 123.6 and 132.9 (2C, CH=CH), 174.3 (1C, C=O); DEPT-135: (D_2O) δ 13.80 (1C,CH_3), 22.61–31.66 (10C, CH_2), 56.38 and 56.74 (2C, epoxy, CHCH), 123.57 and 132.68 (2C, CH=CH), 56.2 (2C, N(CH_3)$_2$).

Reductive Amination of Vernonia Oil Amides

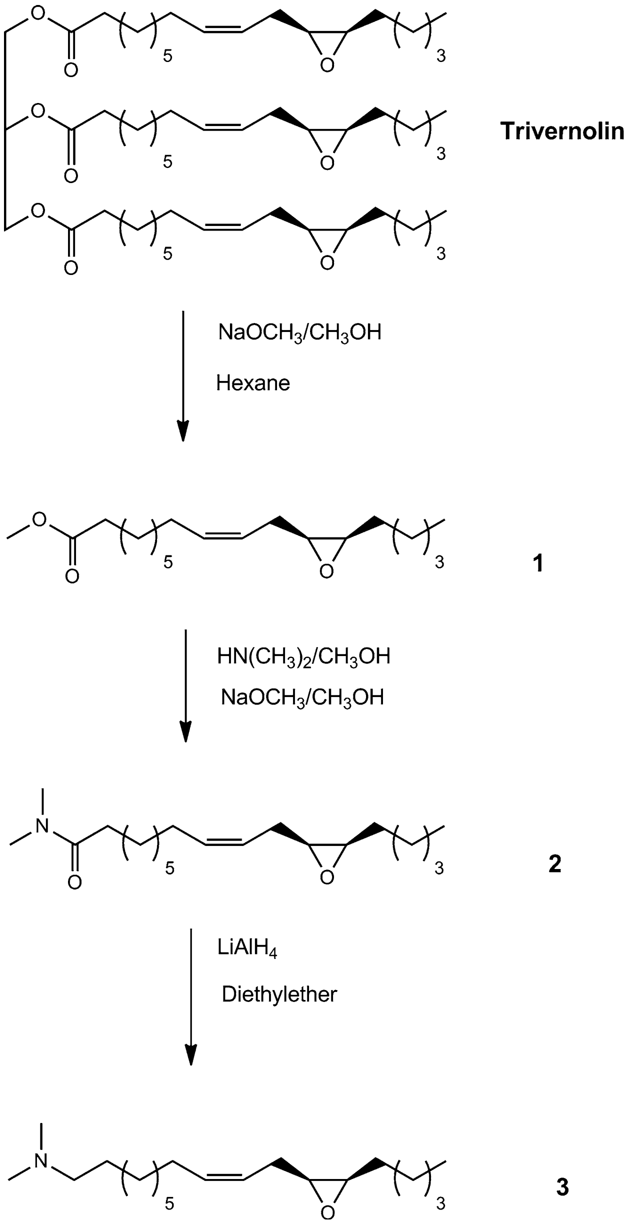
To a 100-mL round-bottom flask equipped with a magnetic stir bar was added the amide mixture containing vernolamide (**2**) (0.513 g, 1.59 mmol), followed by trituration with 20 mL of diethyl ether after which an additional 30 mL of diethyl ether was added. Then LAH (0.12 g, 3.18 mmol) was added slowly, and refluxed for 30 min. The reaction mixture was then allowed to cool to room temperature and placed in an ice-water bath (0 °C) while 20 mL of water was added slowly. The solution was vacuum filtered and the filtrate extracted with two 40-mL portions of diethyl ether. The ether layers were combined and dried with anhydrous sodium sulfate. The ether was evaporated via a rotary evaporator to reveal a light yellow oil 0.26 g (52.1%) of the desired product. GC–MS revealed a mixture of palmitamine, oleamine, stearamine, and major product vernolamine **(3)**. The molecular ion for vernolamine **(3)** was at *m/z* 309 and diagnostic ion of *m/z* 238 [M-71]. ^{1}H: (CDCl$_3$) δ 0.91 (t, 3H, CH_3), 1.25–1.60 (m, 22, 11CH_2), 2.03 (q, 2H, CH_2CH=CH), 2.21 (s, 6H, N(CH_3)$_2$), 3.63 (t, 2H, CH_2N), 2.92 (broad, 2H, epoxy, C*HCH*), 5.53 (m, 2H,C*H*=C*H*); ^{13}C: (CDCl$_3$) δ 14.0 (1C, *C*H$_3$), 22.0-33.0 (11C, *C*H$_2$), 45.5 (2C, N(*C*H$_3$)$_2$), 56.6 and 57.2 (2C, epoxy, *C*H*C*H), 59.9 (1C, *C*H$_2$N), 123.8 and 132.7 (2C, *C*H=*C*H). DEPT-135: (CDCl$_3$) δ 14.01 (1C,*C*H$_3$), 22.60–31.75 (11C, *C*H$_2$), 45.49 (2C, N(*C*H$_3$)$_2$), 56.24 and 57.24 (2C, epoxy, *C*H*C*H), 59.95 (1C, *C*H$_2$N), 62.95 (1C, CHOCH*C*H$_2$ CH=CH), 123.82 and 132.68 (2C, *C*H=*C*H).

Synthesis of N,N,N-Ethyl-dimethyl-(12S,13R)-epoxy-cis-9-octadecenyl Ammonium Bromide (Fig. 2) and Other Quaternary Amines

The amine mixture (0.35 g, 0.001 mol) was refluxed with bromoethane (5 mL, 0.07 mol) for 28 h in ethanol. The solvent was then removed via a rotary evaporator. Next, 20 mL of water was added and the aqueous mixture was extracted with three 15-mL portions of diethyl ether. The water was evaporated in the hood in 24 h to reveal the desired quaternary ammonium salts which were confirmed by MALDI-TOF MS (Fig. 3).

Results and Discussion

The goal and challenge in this synthetic approach was to preserve the integrity of the double bond and epoxy functionalities of the major products, which are expected to show good versatility in the epoxy amine product. The first step in this synthetic approach was the synthesis of VOME from VO which was adapted from a previous method in our lab [18]. The desired methyl ester products were achieved via a based-catalyzed methanolysis in a 98% yield (Scheme 1).



Scheme 1 Synthetic scheme for the conversion of trivernolin, the major TG of vernonia oil, to *N*,*N*-dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenyl amine. Only the major product of each step is shown

From the literature it is known that esters react with amines to form amides [19–21]. With this knowledge we chose to react VOME **(1)** with dimethylamine serving as reagent and solvent in the presence of sodium methoxide as catalyst under reflux conditions for 2 h to afford the amide mixture with major product vernolamide **(2)** (Scheme 1). Before arriving at these optimum reaction conditions, several attempts were made without catalyst, resulting in long reaction times and incomplete conversion of the starting methyl esters to amides. Incorporation of sodium methoxide resulted in shorter reaction times and complete conversion as evidenced by GC–MS monitoring of the reaction. The GC (Fig. 1) showed a mixture in which all methyl esters were converted to their corresponding amides. The optimal molar ratio of ester:dimethylamine:sodium methoxide was 1:1:10. The optimal catalytic amount of sodium methoxide was 0.1 mol. If less was used, incomplete conversion resulted. A larger amount of catalyst showed no distinguishable difference in the yield observed. It is believed that the addition of sodium methoxide increases the nucleophilicity of the amine and therefore promotes the attack of the amine on the carbonyl carbon of the ester. We also investigated reaction time in which aliquots of the reaction mixture were taken for GC–MS analysis every 30 min. Longer reflux times did not show an increase in product yield. The pseudo- molecular ion peak in MS (Fig. 1) for vernolamide **(2)** was at *m/z* 324 (M + H) with diagnostic peaks at *m/z* 210 (M-113) and *m/z* 87 (McLaferty rearrangement). FTIR data showed an absorbance at 1,631.21 cm^{-} which was representative of the carbonyl of an amide. ^{13}C data revealed peaks at 37.4 and 37.8 ppm corresponding to the two methyl carbons attached to the nitrogen in vernolamide and the disappearance of the peak at 51.38 ppm corresponding to the methyl group attached to the oxygen in methyl vernolate. The peaks at 56.6 and 57.3 ppm and 123.6 and 132 ppm corresponding to the double bond and the epoxy, respectively, still remained, indicating the presence of these functionalities. No additional peaks were seen corresponding to epoxy ring opening. This was further confirmed with DEPT-135 NMR.

Synthesis of the desired tertiary amines including vernolamine **(3)** (52.15%), was by the reduction of the vernonia oil amides synthesized in the previous step with lithium aluminum hydride with diethyl ether as solvent. LAH was the second choice of reducing agent after first trying BH_3-THF which afforded no positive outcome. The reduction using BH_3-THF as reducing agent was monitored with GC–MS but after 18 h of reflux only amide starting material was seen and thus the optimal conditions for this route of reduction were not explored. LAH as reducing agent, was employed in a ratio of 1:2 (amide:LAH). Total conversion of the amides to amines was confirmed by GC–MS. Analysis of the product by GC–MS showed a mixture of palmitamine, oleamine, stearamine, and the predominant amine vernolamine **(3)** whose molecular ions were ***m/z* 269, *m/z* 295, *m/z* 297, and *m/z* 309 respectively. There was also a small amount of epoxy ring opening product as evidenced by a small peak with molecular ion at *m/z* 311. This finding was also confirmed by MALDI-TOF MS (Fig. 2). FTIR data revealed the

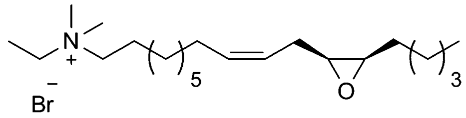


Fig. 2 Structure of *N*,*N*,*N*-ethyl-dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenyl ammonium bromide synthesized from the amine mixture containing the major product *N*,*N*-dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenylamine and ethyl bromide in ethanol under reflux conditions

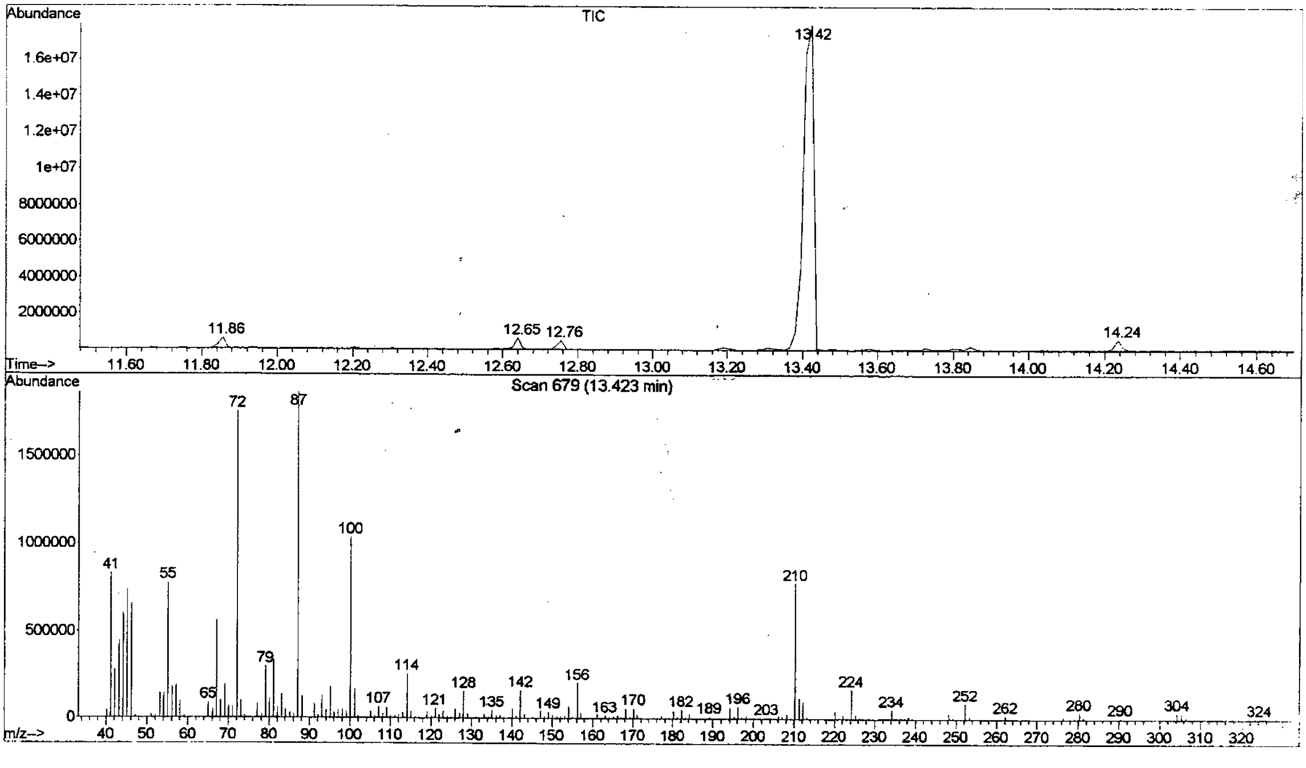


Fig. 1 Gas chromatogram of the amide mixture, and mass spectrum of *N*,*N*-dimethyl-(12*S*,13*R*)-epoxy-*cis*-9-octadecenyl amide (vernolamide). The chromatogram shows four peaks corresponding to palmitamide (r.t. 11.86), oleamide (r.t. 12.65), stearamide (r.t. 12.76), and vernolamide (r.t. 13.42) and an unknown impurity at r.t. 14.24. The mass spectrum of vernolamide shows a pseudo-molecular ion at *m/z* 324 (M + H) and diagnostic peaks at *m/z* 210 (M-113) and *m/z* 87 (McLaferty rearrangement)

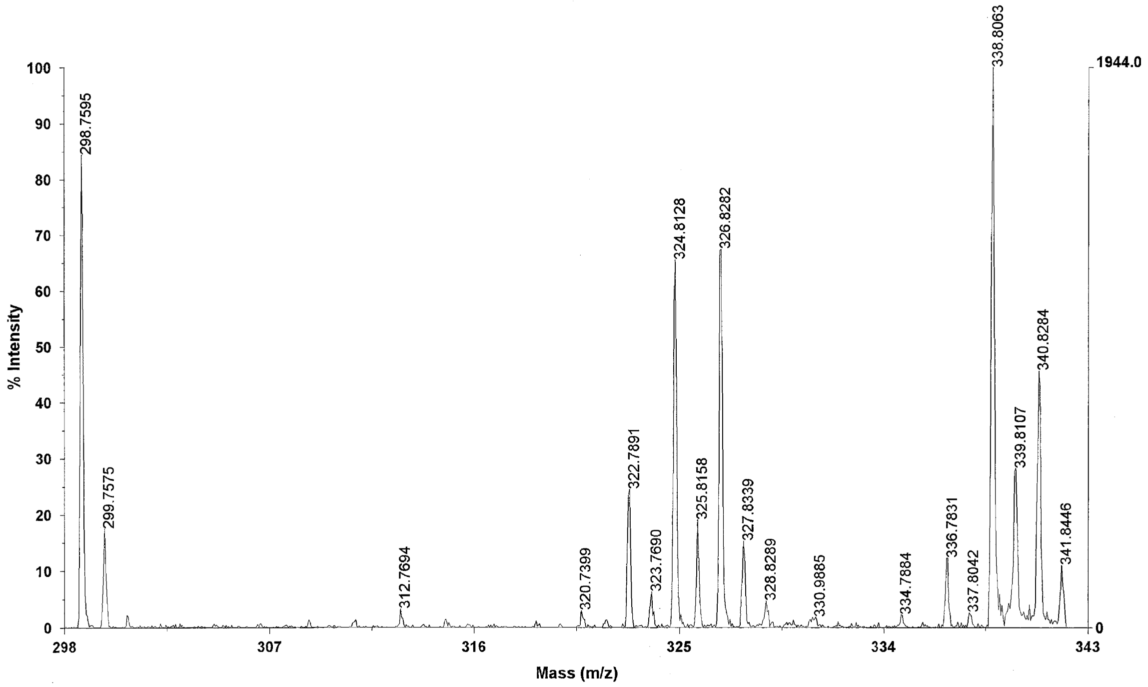


Fig. 3 MALDI-TOF mass spectrum of the mixture of quaternary ammonium salts which contained palmityl (*m*/*z* 298), linoleyl (*m*/*z* 322), oleyl (*m*/*z* 324), stearyl (*m*/*z* 326) and vernolyl (*m*/*z* 338) along with their corresponding isotopic peaks. The peak corresponding to *m*/*z* 340 is attributed to a small amount of the epoxy ring opening product

disappearance of the amide carbonyl absorbance which was expected. ^{13}C NMR spectrum showed the appearance of the methylene carbon attached to the nitrogen at 59.9 ppm and the disappearance of the peak at 174.3 ppm which was representative of the amide carbonyl. Also still present were the peaks representing the double bond and epoxy carbons. DEPT-135 NMR confirmed these findings.

To further confirm the presence of the tertiary amine products, their quaternary ammonium salts were also synthesized and characterized by MALDI-TOF (Fig. 3). MALDI revealed a mixture of palmitic, oleic, linoleic, stearic, and vernolic quaternary salts with the predominant salt being vernolic, corresponding to peaks at *m*/*z* 298, *m*/*z* 322, *m*/*z* 324, *m*/*z* 326, and *m*/*z* 338, respectively, as well as their isotopic peaks (Fig. 3). The ion at *m*/*z* 340 was attributed to epoxy-ring opening.

In summary, this study presents a methodology for the synthesis of a mixture of tertiary amines from vernonia oil with N,N-dimethyl-(12*S*,13*R*)-epoxy-cis-9-octadecenyl amine **(3)**, a functionalized tertiary amine, as the major product. The physical properties and application studies of the products are still being investigated. The quaternary ammonium salts were also synthesized for characterization purposes. By maintaining the epoxy and double bond functionalities of the major product, we open up new avenues to functionalized oleochemicals and surfactants starting with vernonia oil.

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References

1. Perdue RE (1988) Systematic Botany in the development of *Vernonia galamensis* as a new potential oilseed crop for the semi arid tropics. Sym Dot Ups 28:125
2. Ayorinde FO, Butler BD, Clayton MT (1990) *Vernonia galamensis*: a rich source of epoxy acid. J Am Oil Chem Soc 67: 844–845
3. Ncube I, Read JS, Aldercreutz P, Mattiasson B (1998) Triacylglycerols of vernonia galamensis seed oil. Phytochemistry 47: 723–727
4. Pryde EH (1979) Vegetable oil raw materials. J Am Oil Chem Soc 56:719A
5. Carlson KD, Chang SP (1985) Chemical epoxidation of a natural unsaturated epoxy seed oil from *vernonia galamensis* and a look at epoxy oil markets. Ibid 62:934–939
6. Baye T, Becker HC, Witzke-Ehbrecht S (2005) *Vernonia galamensis*, a natural source of epoxy oil: variation in fatty acid composition of seed and leaf lipids. Ind Crops Prod 21:257–261
7. Negm NA, Zaki MF, Salem MAI (2010) Cationic schiff base amphiphiles and their metal complexes: surface and biocidal activities against bacteria and fungi. Colloids Surf B 77:96–103
8. Menger FM, Catlin KK, Chen XY (1996) Synthesis and properties of nine new polyhydroxylated surfactants. Langmuir 12: 1471–1473
9. Hamid M, Williams J (2007) Ruthenium-catalyzed synthesis of tertiary amines from alcohols. Tetrahedron Lett 48:8263–8265
10. Moore JL, Taylor SM, Soloshonok VA (2005) An efficient and operationally convenient general synthesis of tertiary amines by

direct alkylation of secondary amines with alkyl halides in the presence of Huenig's base. ARKIVOC 6:287–292

11. Akamatsu H, Kusumoto S, Fukase K (2002) Reactivity switching on solid support: synthesis of tertiary amines by reduction of tertiary amides with $LiAlH_4$. Tetrahedron Lett 43:8867–8869
12. Wang R, Gregg BT, Zhang W, Golden KC, Quinn JF, Cui P, Tymoshenko (2009) Rapid Ti (Oi-Pr)4 facilitated synthesis of α,α,α-trisubstituted primary amines by the addition of grignard reagents to nitriles under microwave heating conditions. Ibid 50:7070–7073
13. Jordan EF, Palm WE, Port WS (1961) Polymerizable derivatives of long-chain fatty alcohols. III. Copolymers of some alkyl acrylates and acrylonitrile. J Am Oil Chem Soc 38:231–233
14. Meier MAR (2009) Metathesis with oleochemicals: new approaches for the utilization of plant oils as renewable resources in polymer science. Macromol Chem Phys 210:1073–1079
15. Doll KM, Sharma BK, Erhan SZ (2008) Friction reducing properties and stability of epoxidized oleochemicals. Clean 36:700–705
16. Watanabe S, Fujita T, Sakamoto M (1988) Epoxides of unsaturated fatty acids as anti-rust additives in water-based cutting fluids. J Am Oil Chem Soc 65:1311–1312
17. Park S, Jin F, Lee J (2004) Effect of biodegradable epoxidized castor oil on physicochemical and mechanical properties of epoxy resins. Macromol Chem Phys 205:2048–2054
18. Elhilo EB, Anderson MA, Ayorinde FO (2000) Synthesis of cis-12,13-epoxy-cis-9-octadecenol and 12(13)-hydroxy-cis-9-octadecenol from vernonia oil using lithium aluminum hydride. J Am Oil Chem Soc 77:874–878
19. Huang P, Zheng X, Deng X (2001) DIBAL-H-H_2NR and DIBAL-H-HNR^1R^2•HCl complexes for efficient conversion of lactones and esters to amides. Tetrahedron Lett 42:9039–9041
20. Machetti F, Bucelli I, Indian G, Kappe CO, Guarna A (2007) Parallel synthesis of an amide library based on the 6, 8-dioxa-3-azobucyclo[3.2.1] octane scaffold by direct aminolysis of methyl esters. J Comb Chem 9:454–461
21. DeFeo RJ, Strickler PD (1963) An improved method of synthesis of secondary amides from carboxylic esters. J Org Chem 28: 2915–2917