

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES



GRADUATE PROJECT (Chem. 774)

SYNTHESIS OF CARBOHYDRATE ESTERS
FROM VERNONIA GALAMENSIS
SEED OIL.

BY: ADDISU ALEM

October 3, 2010

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FROM
VERNONIA GALAMENSIS SEED OIL
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DEPARTMENT OF CHEMISTRY

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LIST OF SYMBOLS AND ABBREVIATIONS USED

VO.....	Vernonia Oil
IPN.....	Interpenetrating Polymer Networks
PVC.....	Polyviyl Chloride
FFA.....	Free Fatty Acids
VOME.....	Methyl Vernolate
AGU.....	Anhydroglucose Unit
NMR.....	Nuclear Magnetic Resonance
ppm.....	Parts Per Million
δ (delta).....	the symbol used to indicate chemical shift value
m.....	multiplet

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ABSTRACT
SYNTHESIS OF CARBOHYDRATE ESTERS
FROM
VERNONIA GALAMENSIS SEED OIL

Vernonia galamensis is one of only a few plants containing naturally occurring epoxy oils in its seeds known as vernonia oil. Seeds of *vernonia galamensis* produce triglyceride oil rich in trivernolin that can be converted into vernolic acid (72 - 80% of the seed oil).

Carbohydrates are the most abundant organic compounds on the planet. They constitute a suitable replacement for fossil fuels since they contain a considerable amount of carbon and hydrogen. They are renewable natural resources, which are widespread and inexpensive, and from which a wealth of bulk of fine chemicals can be produced.

The derivatives of carbohydrates with fatty acids have the potential to give value-added products and highly versatile materials with interesting characteristics. These compounds, derived from natural oils and carbohydrate, are used as surfactants in the food and cosmetic industries, as insecticides and antimicrobial agents.

V. galamesnsis seed oil was first extracted, and methyl vernolate and fatty acid chloride were synthesized and allowed to react with carbohydrates such as glucose and cassava starch and the products were characterized using NMR.

Keywords: *Vernonia galamensis*, methyl vernolate and amylopectin structure.

Introduction

1.1. *Vernonia Galamensis* Plant

Vernonia (*Vernonia galamensis*) is potentially novel industrial oilseed crop. The genus *vernonia* is one of the largest groups in the family of Asteraceae. According to the species *galamensis* is recognized to include six subspecies including *galamensis*, *mutomoensis*, *nairobensis*, *afromomntana*, *gibbosa*, and *lushotoensis*. The sub-species *galamensis* and *mutomoensis* are found in areas that receives as little as 200 mm rainfall per year. Higher elevations and areas of high rainfall are the suitable regions for the sub-species *afromontana* and *lushotoensis* ^[4]. Sub-species *galamensis* is the most diverse and widely distributed with 4 botanical varieties: *galamensis*, *petitiana*, *australis* and *ethiopica* ^[1].

Vernonia galamensis is limited in distribution primarily to Eastern Africa. This plant grows in a wild form in Eritrea, Ethiopia, Malawi, Tanzania and Kenya. It has been found to grow in areas with less than 600mm rainfall and thrives in sandy soils ^[2].

V. galamensis subsp. *galamensis* variety *ethiopica* was first identified by Perdue in 1964 in Eastern Ethiopia along the Harar-Jijiga road at 9°14' N and 42°35' E, 1700 m above sea level ^[4]. Later, south and south-eastern Ethiopia was described as a natural habitat of this botanical variety. List of geographical location and coordinates of *vernonia* (*V. galamensis* subsp. *galamensis*) varieties in Ethiopia are ^[1]:

- Gelemso (08° 49' N, 40° 31' E)
- Melkabelo (09° 12' N, 41° 25' E)
- Harar Zuria (09° 19' N, 42° 07' E)
- Metta (09° 25' N, 41° 34' E)
- Gelemso (08° 49' N, 40° 31' E)
- Yirgalem (06° 42' N, 38° 21' E)
- Leku (06° 52' N, 38° 27' E)

- Awassa (06° 52' N, 38° 27' E)
- Areka (06° 48' N, 37° 43' E)
- Arsi-Negele (07°00' N, 38° 35' E)



Figure 1: *Vernonia galamensis* plant.

Vernonia galamensis plays a great role in oleochemical industry and alternative cash crop as a primary source of income for farmers. Developing countries can also capitalize on growing it for export or for their own industrial development ^[3, 4].

1.1.1. Vernonia Oil Composition

Vernonia galamensis is one of only a few plants containing naturally occurring epoxy oils in its seeds known as vernonia oil. The best quality vernonia oil is the one in which all of the epoxy acid is present as trivernolin (Figure 2), which is rich in epoxy fatty acids ^[5-7].

Seeds of *Vernonia galamensis* produce triglyceride oil rich in trivernolin (35- 42% of the seed) that can be converted into the acid, which is environment friendly, less expensive and less viscous compared to other artificial epoxy oils ^[3]. Vernolic acid composes 72 - 80% of the acids present in the seed oil. Vernonia oil also contains other fatty acids such as linoleic acid (12 -

14%), oleic acid (4 - 6%), stearic acid (2 - 3%), palmitic acid (2 - 3%) and a trace amount of arachidic acid (Carlson et al., 1981) ^[1-4, 6] (Figure 3).

The natural, liquid epoxy oil form *vernonia galamensis* seed has properties, such as oxirane content (4%), viscosity (110 cps) and molecular weight (926). Furthermore, *vernonia galamensis* seed oil (VO) consists of additional epoxidizable unsaturation, so that fully epoxidized VO could have an oxirane value near 10% ^[1, 8-13].

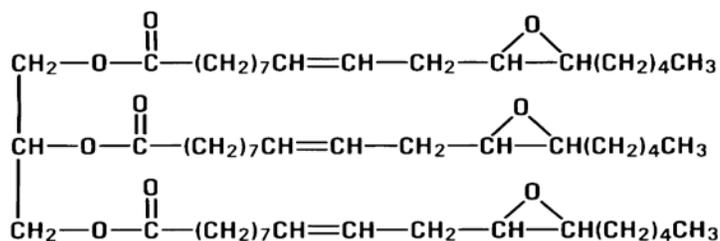


Figure 2: The structure of trivernolin.

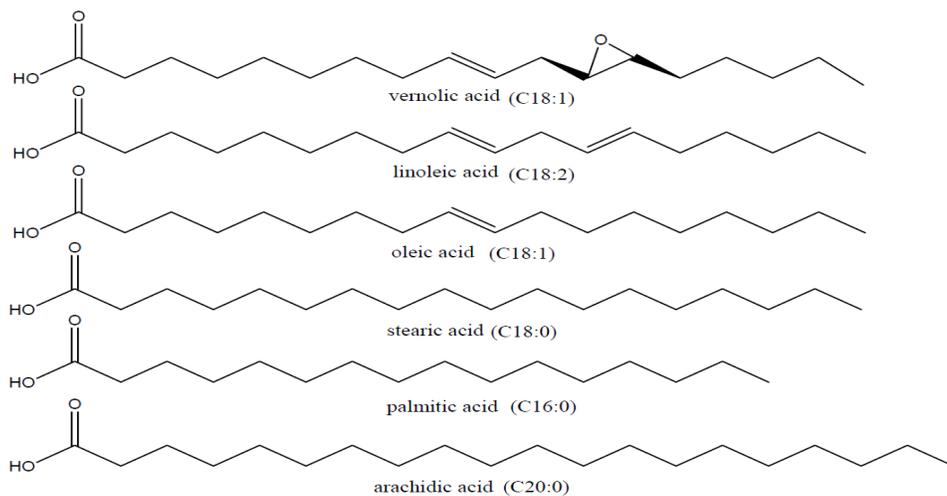


Figure 3: Structures of fatty acids of vernonia oil.

1.1.2. Economic Importance of Vernonia Oil

Several publications have focused on *vernonia galamensis*, a promising source of epoxy-triglycerides and epoxy acids. The commercial importance of this oil includes: a reactive diluent in coatings, in plastic formulations, chemical coatings, adhesives, plasticizers and stabilizers and as a chemical intermediate, thus giving the species great economic importance ^[14].

The presence of epoxy group, the low viscosity and polymerizing characteristics of this oil makes it valuable as a solvent in industrial coatings and paints. Some of the products that are developed from vernonia oil are also degradable lubricants and lubricant additives, epoxy resins, plastic formulations of poly vinyl chloride (PVC), adhesives, insecticides and insect repellants and reactive monomers in polymer synthesis [5-7, 16].

Other applications of the product that developed from vernonia oil are for the synthesis of polyurethane foams and IPN, as the pH stabilizers, waxes, glues, emulsifiers and rust suppressions, and in organic formulations of carriers for low-release pesticides and herbicides. Vernonia oil has also been used as a source of hydroxyl alkoxy fatty esters and for the synthesis of epoxy secondary amides [17]. Currently vernonia product on the market includes Vernola Super Gloss, a car-care product used on tires, leather, and rubber bumpers [14-15].

1.1.3. Reactivity of Vernonia Oil

The unique and special structure of epoxy acid within the triglyceride enables a wide variety of reaction characteristics of the ester group, the double bond, and the epoxy group to occur as shown (Figure 4) [6].

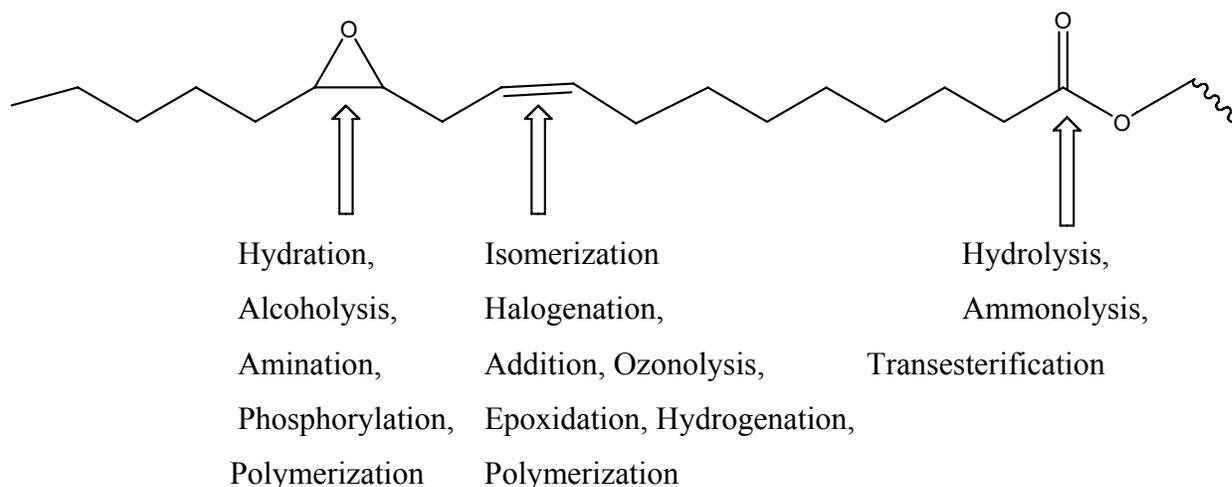


Figure 4: A wide variety of reaction characteristics of epoxy acid within the triglyceride.

Vernolic acid group serves as a key starting material for the synthesis of different important chemicals. For instance bombykol (which has been identified as a sex pheromone of the silk

worm *Bombyx mori* and can be used in pest control) is synthesized from vernolic acid. In addition, traumatic acid (which is active as wound hormone of plants) is synthesized from vernolic acid derived intermediate ^[18].

Synthesis of the acrylate and methacrylate monomers can be obtained by reacting vernonia oil with acrylic or methacrylic acid, with high conversion of the epoxy group. The obtained monomers were polymerized by sunlight. Thereafter, IPNs were prepared from the sunlight-cured methacrylate of vernonia oil as the elastomeric component in combination with a cured bisphenol A-type epoxy resin. The ecological advantages of poly-functional acrylate monomers are further enhanced by the fact that sunlight may be used as the curing agent in the production of highly cross-linked polymers ^[15].

The synthesis of a toughened elastomer from vernonia *galamensis* seed oil by reacting vernonia oil with vernonia oil-derived suberic acid (octanedioic acid), and cross-linking the pre-polymer in the immediate presence of cross-linked polystyrene prepared in situ the synthesized toughened elastomer suggests that vernonia oil-suberic acid polyester and polystyrene polymer are interpenetrating. The utilization of VO as a source of industrially important dibasic acids, was recently reported the synthesis of a series of dibasic acids from VO ^[11, 19].

The carbonated vernonia oil retains the characteristics of low viscosity of vernonia oil, and offers potential as valuable, bio-based intermediate for synthesizing low viscosity resins. Esters of carbonic acid (H_2CO_3) are increasingly used as solvents and reactive intermediates and in metal ion extraction. More attractive method of synthesizing carbonates is by reacting carbon dioxide with an epoxide ^[20].

1.2. Carbohydrates

Carbohydrates are the most abundant and widely distributed, naturally occurring compounds on earth. They are the product of photosynthesis, storing light energy in the form of chemical energy. Their general formula is $C_n(H_2O)_n$. They are polyhydroxy, aldehydes or ketones which can be reduced to give sugar alcohols, oxidized to give sugar acids, substituted at one or more of the hydroxyl groups to give other compounds or derivatized at the hydroxyl groups.

Carbohydrates constitute a suitable replacement for fossil fuels since they contain a considerable amount of carbon and hydrogen. They are a renewable natural resource which is widespread and inexpensive, and from which a wealth of bulk and fine chemicals can be produced.

The poly-functionality allows for many products to be obtained from even the simplest reaction. Different degrees of substitution result in different physicochemical properties, which in turn will be vital to different applications. In order to control the degree of substitution, the regioselectivity of the sugar should be well understood. Depending on the reaction conditions and catalysts, the transformation can be oriented towards many different positions.

Green biodegradable polymers derived from natural resources are potentially very interesting substitutes for non-biodegradable petroleum-based polymers. An attractive field of application for these polymers is the use as packaging materials. For the current petrochemical based products recycling is often neither practical nor economically feasible. Natural polymers such as starch, cellulose or proteins are potentially very interesting starting materials for biodegradable packaging materials. In particular starch is attractive as it is relatively cheap and abundantly available. However, the use of native starch for packaging materials is limited due to its low moisture resistance, high viscosity, high brittleness, and incompatibility with hydrophobic polymers. Further modification of starch is therefore required to introduce hydrophobicity and to improve mechanical and moisture barrier properties.

1.2.1. Glucose

Glucose is a carbohydrate found in many materials. It is present in combined forms in cellulose, starch, sucrose, lactose. It may also be found in the free-state in materials such as honey or grapes. In the body, the cells use it as a source of energy and metabolite intermediate.

Glucose is a monosaccharide which contains six carbon atoms and an aldehyde group. It can exist in an open-chain (acyclic) and ring (cyclic) form (Figure 5 and 6), the latter being the result of an intramolecular reaction between the aldehyde carbon atom and the C-5 hydroxyl group to form an intramolecular hemiacetal. In an aqueous solution, both forms are in equilibrium, and at pH 7 the cyclic one is the predominant. The ring contains 5 carbon atoms and one oxygen atom, and resembles the structure of pyran. In this ring, each carbon is linked to hydroxyl side group

with the exception of the fifth atom, which links to a sixth carbon atom outside the ring, forming CH₂OH group.

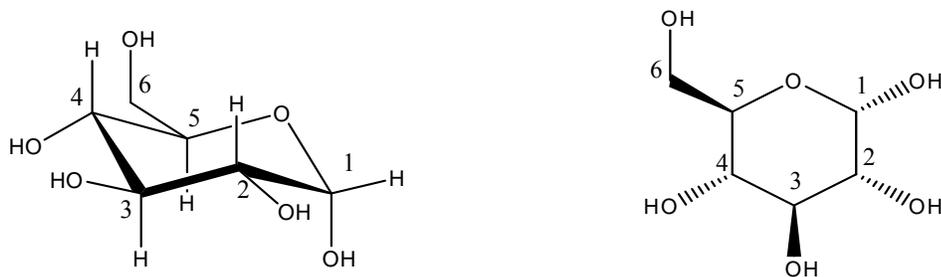


Figure 5: The structures of α -Glucose.

When glucose is in its ring form, an additional asymmetric carbon, the anomeric carbon atom, is created at C-1. This leads to the formation of two ring structures, the anomers α -Glucose and β -Glucose. In the α form, the hydroxyl group attached to C-1 is below the plane of the ring, in the β form it is above. The α and β forms interconvert over a timescale of hours in aqueous solution, to a final stable ratio of $\alpha : \beta$ 36:64, in a process called mutarotation (Figure 6).

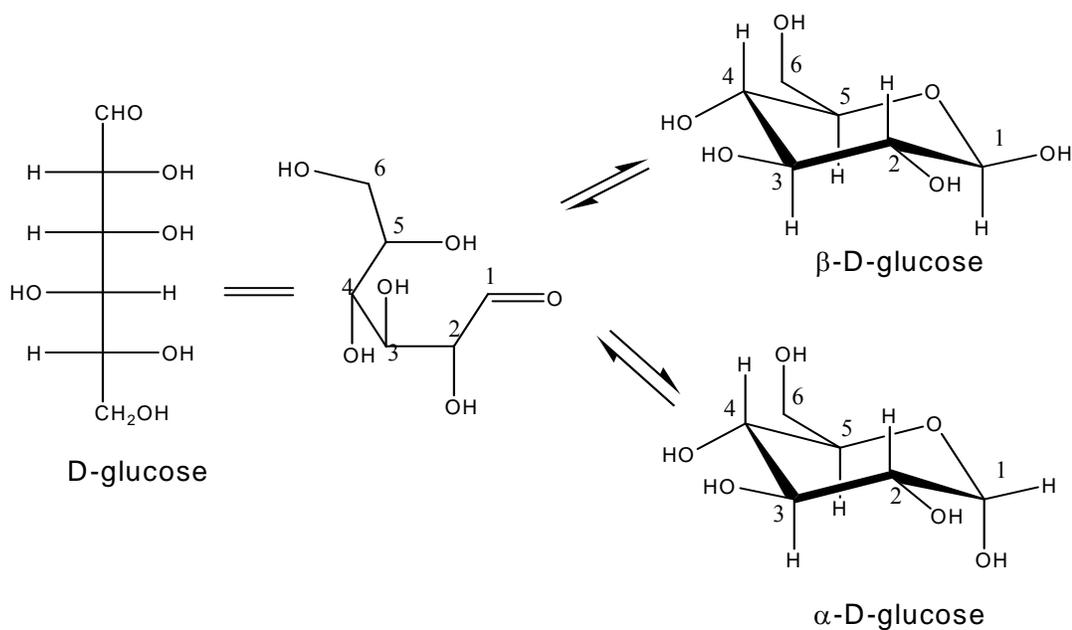


Figure 6: D-glucose ring closure and mutarotation.

1.2.2. Starch

Starch (figure 7) is an abundant, inexpensive, renewable, and fully biodegradable natural raw material which has generated a renewed interest in recent years. Starch based material may be used as constituents for petroleum-based plastic material especially in the packaging industries. Since starches are biodegradable, so they may offer an alternative solution to the disposal problem of petroleum-based materials [21, 22].

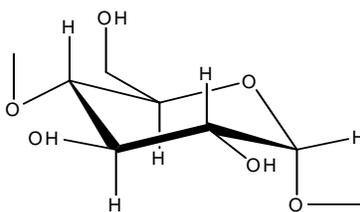


Figure 7: The structure of Starch.

Starch consists of two primary polymers containing d-glucose, namely the linear α -(1 \rightarrow 4) linked amylose and the amylopectin that is composed of α -(1 \rightarrow 4) linked d-glucose and α -(1 \rightarrow 6) linked branches (Figure 8). The molecular mass of amylose is in the range 105–106, while amylopectin shows significantly higher values of 107–108. Amylose and amylopectin occur in varying ratios depending on the plant species.

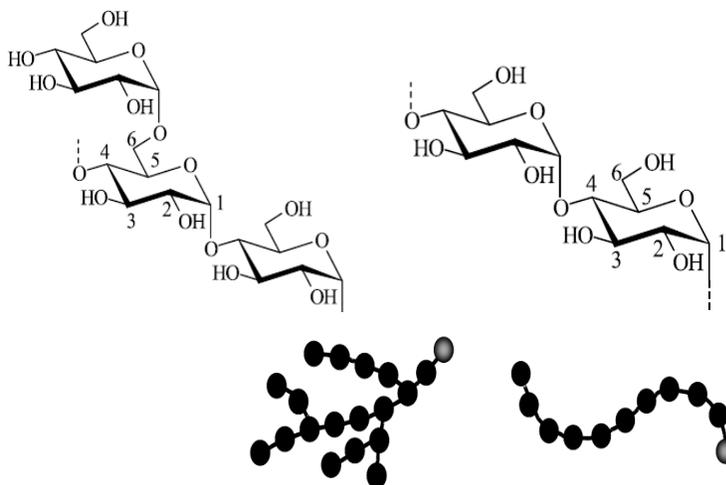


Figure 8: Structures of amylopectin (left) and amylose (right) and schematic representation of the branching pattern.

Cassava starch is one of the most important sources starch given its ease of extraction and high purity with less protein and other associated compounds.

1.3. Sugar Esters of Fatty Acids

Synthesis of carbohydrate esters from natural oils and starch has the potential to give value-added products and highly versatile materials with interesting characteristics. These compounds, derived from natural oils and sugars, are used as surfactants in the food and cosmetic industries, as insecticides and antimicrobial agents, and even as non-caloric fat substitutes. This wide range of applications is made possible by the variety of available sugars and fatty acids. In fact, the properties of these esters are intimately related to their structure.

Replacement of the petroleum based plastics with materials from agro- resources, especially starch, is attractive from the stand point of providing biodegradation properties to the end product. This is used to conserve our petrochemical resources and to find out new non-food uses of starch. Indeed, starch is inexpensive is totally biodegradable, and is available in large quantities from certain crops produced in abundance beyond available markets ^[23].

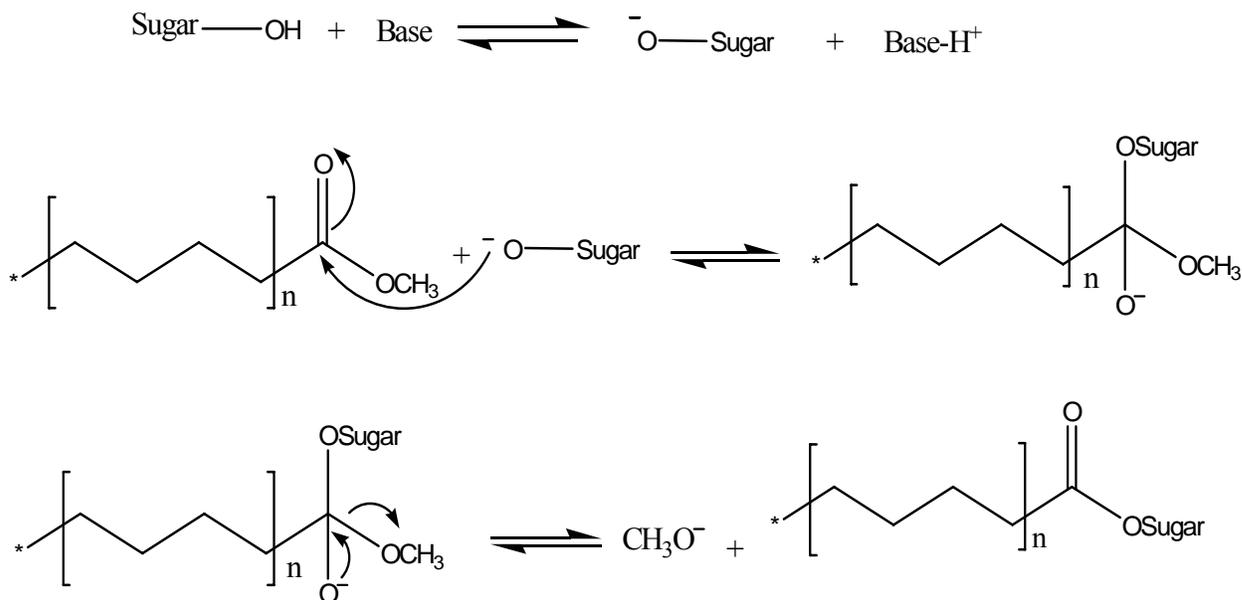
Sugar fatty acid esters, usually called sugar esters, are non-ionic and biodegradable surfactants that have very good emulsifying, stabilizing, or conditioning effects. They are widely used in the food, cosmetic, pharmaceutical, and detergent industries ^[23].

Sugar fatty acid esters with degrees of substitution of 1 to 3 are nonionic, digestible, absorbable, and biodegradable detergents of low toxicity ^[24, 25]. They are used as non-ionic surfactants, bleaching boosters and food additives. Sucrose esters of fatty acid with a low degree of substitution can be used as food and cosmetic emulsifiers. Also, sugar polyesters have applications as fat substitutes. Olestra® (Figure 9), developed by Procter and Gamble, is a polysubstituted fatty acid ester used as a non-caloric fat. It presents similar properties to those of triglycerides but is non-digestible. As the degree of substitution increases, hydrolysis by lipolytic enzymes decreases due to steric hindrance and the type of fatty acid substituted ^[25, 26].

Sugar esters are synthesized by esterification of sugars or sugar alcohols with fatty acids. The esterification process can be carried out either chemically or enzymatically. The chemical process occurs with a low selectivity and leads to a mixture of sugar esters with different degrees

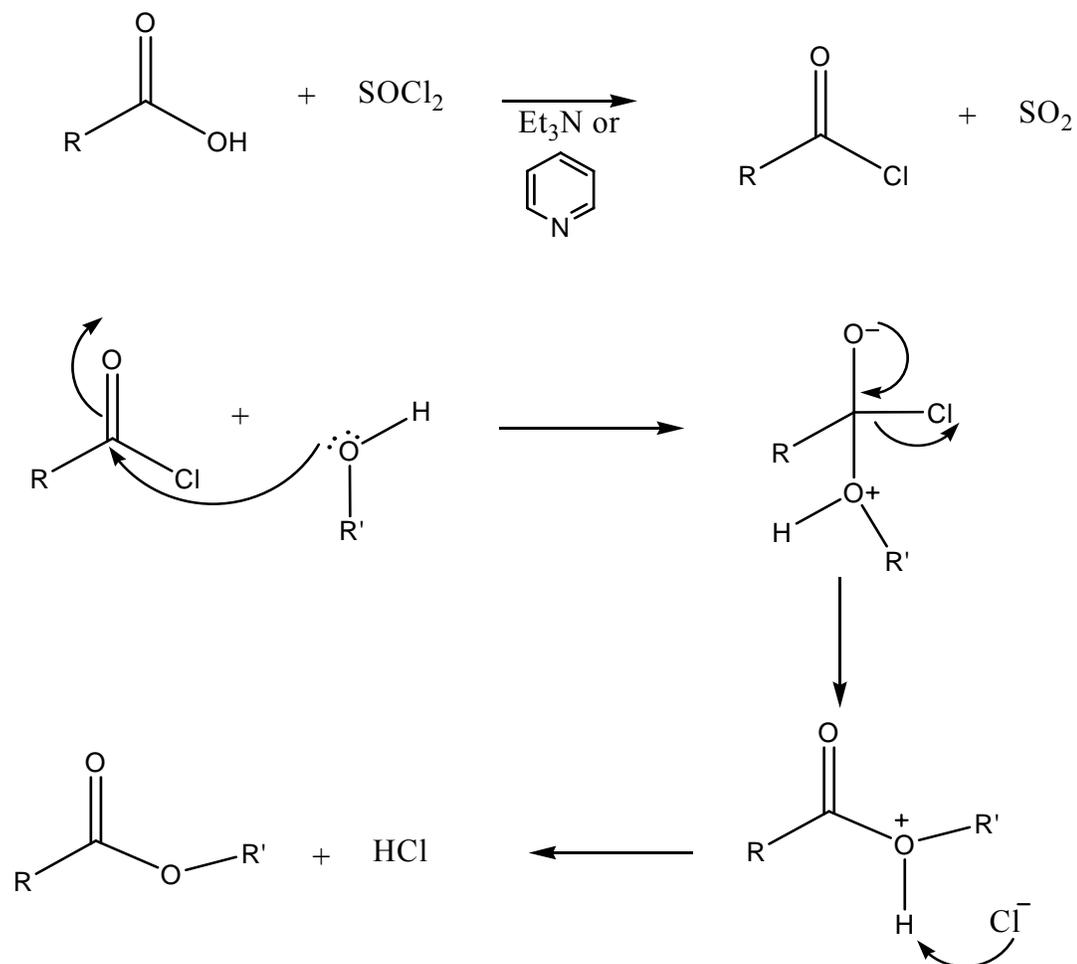
of esterification. It requires toxic organic solvents and is carried out at high temperatures, which causes coloration of the final products. These problems can be overcome by the use of a biological catalyst, such as lipase, for the synthesis of sugar esters [27].

The main advantage of enzymatic synthesis is that its high regioselectivity leads mainly to monoester production. In addition, the enzymatic method can be performed under mild reaction conditions; thus, denaturation of substrate and/or products can be avoided [27].



Scheme 1: Esterification mechanism of fatty acids with sugar.

Sugar esters of higher fatty acid chain lengths can be prepared using acyl chloride method. In this process, HCl is removed by vacuum distillation. Acyl chloride is prepared by treating the carboxylic acid with thionyl chloride (SOCl_2) in the presence of base. Acyl chlorides are the most reactive of the carboxylic acid derivatives and therefore can be readily converted into other carboxylic acid derivatives. Esterification occurs by nucleophilic addition/elimination. The acyl chloride reacts with the alcohol to produce the ester and HCl. The reaction mechanism is as follows:



Scheme 2: Esterification mechanism of fatty acid chloride with sugar.

Starch esters and glucose esters are generally prepared by reacting starch with fatty acid chlorides in organic solvents such as pyridine, toluene, dimethylformamide, or mixtures of lithium chloride and N, N-dimethylacetamide. These methods allow almost complete derivatization of starch. However, organic solvents are toxic and difficult to remove from starch, thus this hindered the use of starch esters in food and pharmaceutical products ^[28].

1.4. Applications of Sugar Esters of Fatty Acids

1.4.1. Emulsifying Properties

The surface active properties of sucrose esters are derived from the original hydrophilic group of sucrose and the original lipophilic group of fatty acids. By varying the degree of substitution or the fatty acid chain lengths, wide ranges of functionality can be obtained ^[24].

Monoesters are soluble in many organic solvents but only slightly in water. Moreover, surfactant properties depend on the degree of esterification as well as the fatty acid chain length and degree of saturation. Sucrose esters with a shorter, more unsaturated fatty acid and less esterified groups show a more hydrophilic function. On the other hand, sucrose polyesters are not good surfactants of oil and water emulsions. However, they are excellent stabilizers of water-oil emulsions because of their lipophilic nature.

1.4.2. Antimicrobial Properties

The antimicrobial activity of sucrose esters comes from the interaction of the esters with cell membranes of bacteria, causing autolysis. The lytic action is assumed to be due to stimulation of autolytic enzymes rather than to actual solubilization of cell membranes of bacteria. The antimicrobial activity of the sugar esters is determined by the structure of the esterified fatty acids. Monoesters are more potent than polyesters and diesters towards gram-positive bacteria.

1.4.3. Fatty Acid Polyesters

When four or more fatty acids are esterified onto sucrose, the polyester behaves as a fat and has physical and organoleptic properties similar to those of fats. Because of a large number of fatty acids are attached to the central sugar molecule, the digestive enzymes can't get in place to break them off and the absorption is slow. Sucrose polyesters are not hydrolyzed by pancreatic lipase and not absorbed in the small intestine. Their characteristics are similar to those of conventional oils but they do not contribute any significant calories. Olestra (Figure 9), developed by Procter and Gamble, is synthetic, non-absorbable sugar polyester, approved by the U.S. FDA for use in snacks as a fat substitute ^[24].

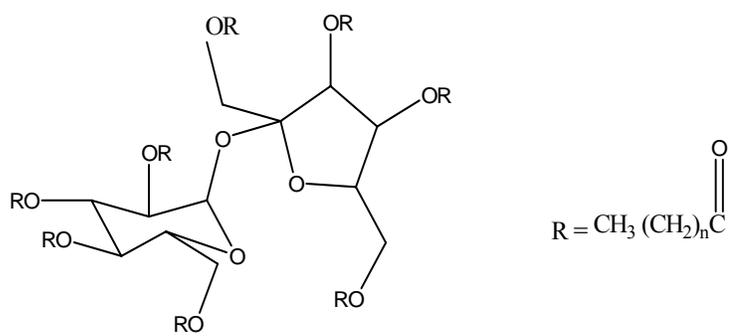


Figure 9: The structure of Olestra®.

2. Objectives of the Study

Since synthesis of carbohydrate esters from natural oils, and glucose and starch has different potential applications with interesting characteristics. These compounds, derived from natural oils and carbohydrates, are used as surfactants in the food and cosmetic industries, as insecticides and antimicrobial agents, and even as non-caloric fat substitutes.

The aim of the project is to synthesize biodegradable carbohydrate esters of fatty acids derived from the naturally epoxidized *Vernonia galamensis* seed oil (trivernolin) and carbohydrates of glucose and starch. Especially, from endemic starch cassava.

3. Experimental

3.1. Materials and Methods

The materials used in the experiment: extraction of the *V.galamensis* seeds was carried out using Soxhlet extraction systems, ¹HNMR and ¹³CNMR were recorded on a Bruker 400 MHz spectrometer with TMS as internal standard and melting points were measured using SMP₃.

The chemicals used in the experiment were: *Vernonia galamensis* seeds, n-hexane, NaOH, Na₂CO₃, NaOCH₃, 85% KOH, Methanol, D-glucose, Casava Starch, glacial acetic acid, pyridin, thionyl chloride, DMSO, K₂CO₃, activated charcoal.

3.2. Extraction and Purification of Vernonia Oil

3.2.1. Extraction of Vernonia Oil

Dried *V. galamensis* seeds were heated in an oven for 1 hour at 90⁰C for lipase deactivation and powdered seeds of *V. galamensis* were extracted with n-hexane as a solvent for three hours using Soxhlet extraction systems. Then the solvent was removed using rotary evaporator and the crude oil was subjected to refining process ^[28, 29].

3.2.2. Refining of Vernonia Oil

In addition to triglycerides, the crude oil contains variable amounts of objectionable substances, which must be removed to produce pure vernonia oil. Some of these impurities are of the non-glyceride type such as the FFA, which may be built up due to enzymatic processes (lipase) resulting from damage to the seed. While others are of the non- oil kind such as the mucilage volatile including moisture and solvent, pigment or coloring materials primary and secondary oxidation products, waxes and saponifiable and odoriferous materials ^[13, 15].

3.2.2.1. Bleaching

Crude vernonia oil was refined with 5% to 8% by weight of activated charcoal was mixed with the oil and heated at a temperature of 60⁰C by a continuous stirring for one hour. The decolorized oil was isolated by hot filtration.

3.2.2.2. Degumming

Almost all seed oils contain impurities in the colloidal state or dissolved in them. These substances must be removed from the oil. This purification process is known as degumming and is usually carried out immediately before neutralization or concurrently with it. Crude vernonia oil was degummed by stirring with 2.5 – 5 g by weight of distilled water, heated 60-70⁰C for one hour followed by centrifugation at 5,000 rpm. Gum and oil were separated and the oil was dried at 60⁰C on a rotary evaporator.

3.2.2.3. Neutralization

Organic acids, which are always dissolved in the oil, are removed by saponification with sodium hydroxide solution. Separations occur easily because the resulting soaps are practically insoluble in the neutral oil under standard operation conditions. The degummed vernonia oil was mixed with sodium hydroxide solution and then heated to 40⁰C followed by stirring for 30 minutes. From the mixer the oil-soap stock suspension passes through the centrifugal separator, which separates the soap stock from the neutral oil. Oil was dried for one hour on a rotary evaporator at 60⁰C.

3.2.2.4. Re-Refining of the Neutral Oil

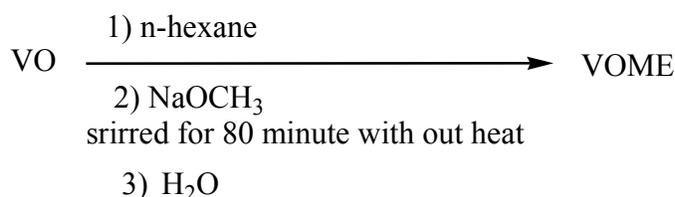
The neutral oil obtained from the neutralization contains minute quantities of free fatty acids (FFAs) and other impurities. Such impurities were removed by treatment with a dilute solution of sodium hydroxide. The purpose of re-refining is to remove the last trace of fatty acids, phosphatides etc. from the neutralized oils.

3.2.2.5. Washing of the Oil

To obtain soap free oil after the degumming, neutralization and re-refining steps, a vigorous washing by hot water was carried out. This is necessary because the soaps are always partially soluble in the neutral oil. Finally, NMR analysis of the purified has been obtained.

3.3. *Synthesis of VOME from Vernonia Oil*

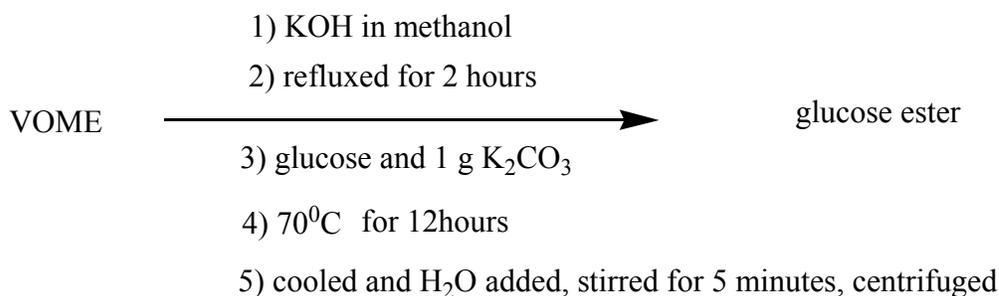
VO (20 g, 64 mmole) was transferred into a 500 mL round-bottomed flask. Hexane (125 mL) was added, followed by 12.5 mL sodium methoxide in methanol (25 wt%). The flask was fitted onto a rotary evaporator, then allowed to rotate (approximately 240 revolutions/min) for about 80 min without heat or vacuum. The resulting mixture was transferred into a separatory funnel and 125 mL water was added. The flask was rinsed with approximately 20 mL water, and the rinse was added to the separatory funnel. The hexane layer was drawn off, and removed by rotary evaporator to result methyl vernolate (VOME) ^[30].



Scheme 3: Synthesis of VOME from VO.

3.4. *Synthesis of Glucose Ester from VOME*

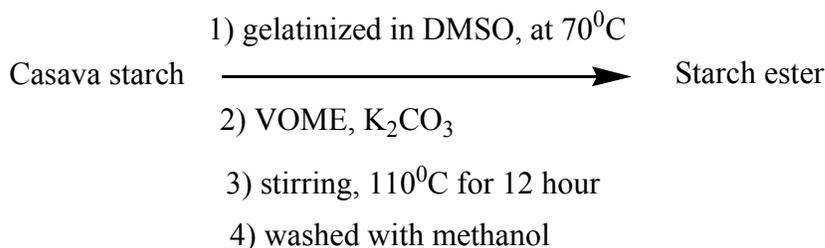
85% KOH pellets (0.055 moles) are dissolved in some methanol and added to VOME (0.347 moles). The mixture is refluxed for 2 hours. At this time, (0.073 moles) of glucose and 1 g K₂CO₃ are added and the condenser is removed. When the reaction reached 70⁰C, reaction conditions are maintained for 12 h. The reaction was cooled, 15 mL of water added, stirred for 5 minutes and centrifuged. The mixture of higher polyesters was then decanted from soap.



Scheme 4: Synthesis of glucose ester from VOME.

3.5. Synthesis of Starch Ester from VOME

Casava starch (2.0 g, 12 mmol, anhydroglucose unit, AGU) was first gelatinized in DMSO (20 mL) at 70⁰C for 3 h. To the solution obtained, VOME (0.036 mol, 11.25 g, (3 mol/mol AGU in starch)) and 0.23 g (2% w/w, with respect to starch) of potassium carbonate (catalyst) were added and the mixture was stirred at 110⁰C for 12 h. After cooling, the product was precipitated using methanol (100 mL) and separated from the liquid phase by decantation. The product was washed twice with methanol (50 and 25 mL, respectively). Finally, the product was dried in a vacuum oven (70⁰C) and a brown solid product was obtained.



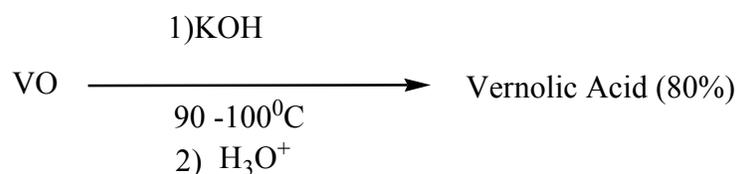
Scheme 5: Synthesis of starch ester from VOME.

3.6. Synthesis of Vernolic Acid from Vernonia Oil

To a 250 mL distilling flask, equipped with magnetic stirrer bar, was transferred 50 mL methanol and 5 g (0.125 mol) sodium hydroxide. The flask was then fitted with a condenser, and the mixture was heated to reflux until complete dissolution of the sodium hydroxide. To the hot alkaline solution 5.12 g (5.5 mmol) VO was added. The resulting brownish solution was refluxed with continuous stirring for 10 minutes, after which it was immediately transferred into a beaker and allowed to form a semisolid on cooling. About 100 g ice was added, mixed thoroughly,

followed by addition of 100 mL water with mixing. The cold mixture was vacuum-filtered to afford an off-white solid soap in the filter bed. The soap was transferred into a beaker and mixed with 100 mg ice and 100 mL water, then acidified with 4 mL glacial acetic acid. The acidified mixture was immediately vacuum filtered to afford a white solid acid. The cold white solid was transferred into a beaker, containing 100 mL hexane, with mixing to dissolve the acid, and the resulting mixture was transferred in to a separatory funnel to allow separation of the organic and aqueous phases. The hexane layer was stripped to afford crude vernolic acid.

Purification of the acid was accomplished by low-temperature recrystallizations. Hexane (50 mL) was added to a 150 mL beaker containing the crude vernolic acid, and the beaker was placed in a freezer for 24 hr. The resulting solid was vacuum-filtered and rinsed with an additional 50 mL ice-cold hexane to give pure vernolic acid and characterized by NMR ^[31].

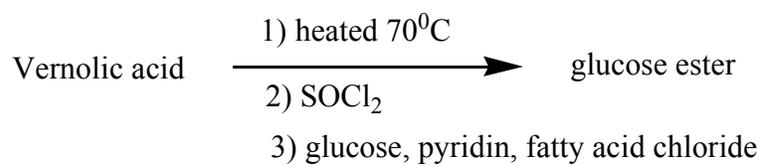


Scheme 6: Synthesis of Vernolic acid from VO.

3.7. Synthesis of Glucose Ester from Fatty Acid Chloride

4.96 grams of vernolic acid (0.019 mmol) were added to a 100 mL flask and heated to 70⁰C in order to obtain a melt. 3 mL of thionyl chloride were then added drop wise and allowed to react for 30 minutes as previously described by Hwang and Fower ^[32]. After this time the excess, unreacted thionyl chloride was removed by rotary evaporation under reduced pressure. The fatty acid chloride was recovered and reacted with glucose as follows:

2.5 g (13.889 mmol) dried glucose was added 15 mL pyridin and 45 g (0.28 mol) fatty acid chloride, and the reaction is heated for 6 h at 115⁰C. The mixture is cooled and poured into 200 mL absolute ethanol, with vigorous stirring. The product is filtered off and washed twice with 200 mL ethanol. Excess ethanol is removed by a rotary evaporator and the glucose ester is dried at 50⁰C overnight.



Scheme 7: Synthesis of glucose ester from Vernolic acid.

4. Result and Discussion

4.1. NMR Analysis of Vernonia Oil

After extraction and necessary purifications steps, the ^1H NMR and ^{13}C NMR spectra of vernonia oil (yellowish colored liquid) obtained showed that the presence of major functional groups namely the ester, the double bonds and the epoxy of triglyceride structure as follows:

^1H NMR data (ppm) (400 MHz, CDCl_3): The ^1H NMR data of the Vernonia oil (Appendix 1 or table 1) shows the presence of olefinic protons ($\text{CH}=\text{CH}$) and glycerol proton (CH) at 5.336 - 5.429, glycerol proton (CH_2) at 4.160 and 4.310, epoxy protons ($\text{O}-\text{C}-\text{H}$) at 2.768 - 2.920, protons of methylene attached to olefinic group ($-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$) at 2.038 - 2.338, protons of methylene groups (CH_2)_n at 1.261-1.627, protons of methyl (CH_3) group at 0.899.

^{13}C NMR data (ppm) (400 MHz, CDCl_3): The ^{13}C NMR data of the Vernonia oil (Appendix 2 or table 2) indicated the presence of the carbonyl carbon ($\text{O}=\text{C}$) at 173.046, olefinic carbons ($\text{CH}=\text{CH}$) at 123.948 - 132.410, glycerol (CH) at 68.900, and glycerol (CH_2) at 62.040-64.998, epoxy carbons ($\text{O}-\text{C}-\text{H}$) at 56.437 - 57.088, methylene carbons (CH_2)_n at 22.599 - 35.421, and methyl carbon (CH_3) at 13.938.

Peak area of different functional groups			
Compounds	Double bond	Epoxy	Methyl ($-\text{CH}_3$)
Crude VO	1	1.1	7.77
Purified VO	1	1	2.34
Theoretical value	1	1	1.5

Table 1: The peak areas of crude VO and purified VO.

The peak area integrations of functional groups of the double bond and the epoxy (table 1) remained the same in crude VO and after purification and both match with the theoretical values.

The purified vernonia oil (VO) is 64% pure containing 34% non-epoxidized trivernolin. This is because of some of the chains from vernolin are not epoxidized ^[33].

4.2. NMR Analysis of VOME

After esterification of vernonia oil, the NMR data for VOME (yellowish color) indicated the presence of major functional groups namely the ester, the double bonds, the epoxy and the methoxy of the VOME structure as follows:

¹H NMR data (ppm) (400 MHz, CDCl₃): The ¹H NMR data of the VOME (Appendix 3 or table 1) indicated the presence of olefinic protons (CH=CH) at 5.223 - 5.376, methoxy protons (O-CH₃) at 3.524, epoxy protons (O-C-H) at 2.651 - 2.775, protons of methylene attached to olefinic group (-CH₂-CH=CH-CH₂-) at 2.166 - 2.172, protons of methylene groups (CH₂)_n at 1.155 - 1.504, protons of methyl (CH₃) group at 0.772.

¹³C NMR data (ppm) (400 MHz, CDCl₃): The ¹³C NMR data of the VOME (Appendix 4 or table 2) showed the presence of the carbonyl carbon (O=C) at 173.647, olefinic carbons (CH=CH) at 123.893 - 132.174, epoxy carbons (O-C-H) at 56.143 - 56.767, (O-CH₃) at 50.968, methylene carbons (CH₂)_n at 22.530 - 33.779, and methyl carbon (CH₃) at 13.862.

Peak area of different functional groups				
Compounds	Double bond	Epoxy	Methyl (-CH ₃)	Methoxy (OCH ₃)
VOME	1	1.08	5.84	1.5
Theoretical value	1	1	1.5	1.5

Table 2: The peak areas VOME.

The peak area integrations of functional groups of the double bond and the epoxy (table 2) fit with the theoretical value and the peak area for the methoxy (OCH₃) is in agreement with the theoretical value which indicates 100% conversion of VO in to vernolic acid.

Generally in the synthesis of VOME from VO, one can observe (Appendix 3 and 4 or tables 4 and 5) clearly the disappearance of glycerol peak of protons and glycerol carbons (CH, CH₂) and the appearance of methoxy (OCH₃) peaks.

4.3. NMR Analysis of Vernolic Acid

After necessary steps the acid obtained (light golden yellow color) from of vernonia oil NMR data indicated the presence of major functional groups namely the ester, the double bonds, the epoxy and the hydroxyl of the vernolic acid structure as follows:

¹H NMR data (ppm) (400 MHz, CDCl₃): The ¹H NMR data of the Vernolic acid (Appendix 5 or table 1) indicated the presence of hydroxylic (-OH) at 10.551, olefinic protons (CH=CH) at 5.321 - 5.482, epoxy protons (O-C-H) at 2.752 - 2.933, protons of methylene attached to olefinic group (-CH₂-CH=CH-CH₂-) at 2.021 - 2.300, protons of methylene groups (CH₂)_n at 1.243-1.601, protons of methyl (CH₃) group at 0.888.

¹³C NMR data (ppm) (400 MHz, CDCl₃): The ¹³C NMR data from the Vernolic acid (Appendix 6, or table 2) showed the presence of the carbonyl carbon (O=C) at 179.782, olefinic carbons (CH=CH) at 123.847 - 132.502, epoxy carbons (O-C-H) at 56.650 - 57.321, methylene carbons (CH₂)_n at 22.577 - 34.268, and methyl carbon (CH₃) at 13.955.

Peak area of different functional groups				
Compounds	Double bond	Epoxy	Methyl (-CH ₃)	Hydroxyl (OH)
Vernolic acid	1	1.06	3.23	0.54
Theoretical value	1	1	1.5	0.5

Table 3: The peak areas Vernolic Acid.

Generally in the synthesis of vernolic acid from VO, one can observe (Appendix 5 and 6 or tables 5 and 6) clearly the disappearance of glycerol peaks of protons and carbons (CH, CH₂) and the appearance of hydroxylic peaks of hydrogen and carbon (O-H).

The peak area integrations of functional groups of the double bond, the epoxy and OH(table 3) match with the theoretical value. But for methyl (CH₃) the peak area integration is 3.23 instead of 1.5. This is because of the presence of non-epoxidized functional groups from VO.

Type s of protons	¹ HNMR Chemical Shift data δ (ppm)		
	VO	VOME	Vernolic acid
Hydroxylic proton (-OH)	-	-	10.551
Olefinic protons (CH=CH)	5.429 – 5.494, m	5.223 – 5.376, m	5.321 – 5.482, m
Glyceral proton (CH)	5.336, m	-	-
Glyceral protons (CH ₂)	4.130 – 4.320, m	-	-
Methoxy protons (O-CH ₃)	-	3.518 – 3.524, m	-
Epoxy protons (-O-C-H)	2.768 – 2.908, m	2.651 – 2.775, m	2.752 – 2.933, m
methylene (-CH ₂ -CH=CH-CH ₂ -)	2.038 - 2.338	2.166 - 2.172	2.021 - 2.300
Methylene groups (CH ₂) _n	1.261 – 1.627, m	1.155 – 1.200, m	1.243 – 1.601, m
Methyl (CH ₃)	0.849 – .9774,	0.767 – 0.772, m	0.865 – 0.888, m

Table 4: ¹HNMR Chemical Shifts of VO, VOME, and Vernolic acid.

Types of carbon	¹³ CNMR Chemical Shift data δ (ppm)		
	VO	VOME	Vernolic acid
Carbonyl carbon (O=C)	173.046	173.647	179.782
Olefinic carbon (CH=CH)	123.948 – 132.410	123.893 – 132.174	123.847 – 132.502
Glyceral (CH)	68.900	-	-
Glyceral (CH ₂)	62.040 – 64.998	-	-
Epoxy carbon (O-C-H)	56.437 – 57.088	56.143 – 56.767	56.650 – 57.321
Methoxy carbon (O-CH ₃)	-	50.968	-
Methylene carbons (CH ₂) _n	22.599 – 35.421	22.530 – 33.779	22.577 – 34.268
Methyl Carbon (CH ₃)	13.938 – 14.051	13.862	13.955

Table 5: ¹³CNMR Chemical Shifts of VO, VOME, and Vernolic acid.

4.4. NMR Analysis of Glucose Ester

¹H NMR data (ppm) (400 MHz, D₂O): The ¹H NMR data of the glucose ester (brown semi-solid, rubber like) (Appendix 7) indicated the presence of hydrogen (C₁) at 8.356, hydrogens of (C₃) at 3.838, hydrogens (C₆, C₅, C₄ and C₂) at 3.496, olefinic protons (CH=CH) at 5.197- 5.430, epoxy protons (O-C-H) at 2.647 - 2.833, protons of methylene attached to olefinic group (-CH₂-CH=CH-CH₂-) at 2.059-2.297, protons of methylene groups (CH₂)_n at 1.164-1.816, protons of methyl (CH₃) group at 0.807.

¹³C NMR data (ppm) (400 MHz, D₂O): The ¹³C NMR data of the glucose ester (Appendix 8) showed the presence of the carbonyl carbon (O=C) at 165.261 - 170.782; carbon (C₁) at 73.431- 74.952; carbons at positions (C₂, C₃, C₄, C₅) at 68.48 - 69.75; carbon (C₆) at 61.41 – 62.545; epoxy carbons (O-C-H) at 57.394 - 58.562, methylene carbons (CH₂)_n at 23.678 - 38.076, and methyl carbon (CH₃) at 17.225 - 20.469.

Generally in the synthesis of glucose ester from VOME, one can observe (Appendix 7 and 8 or tables 5 and 6) clearly the disappearance of methoxy (OCH₃) peak.

Peak area of different functional groups						
Compounds	Double bond	Epoxy	Methyl (-CH ₃)	δ (3.503)	δ (8.354)	δ (8.354)
Glucose Ester	1	0.97	2.44	0.29	0.06	0.06
Theoretical value (1:1)	2	2	3	5	1	1
Theoretical value (1:2)	4	4	6	5	1	1
Theoretical value (1:3)	6	6	9	5	1	1
Theoretical value (1:4)	8	8	12	5	1	1
Theoretical value (1:5)	10	10	15	5	1	1

Table 6: The peak areas Glucose Ester.

The peak area integrations of functional groups of the double bond and the epoxy (table 6) fit with the theoretical value. When we compare the peak area of the double bond and the H at δ (3.503) indicates the substitution of a fatty acid on to glucose molecule is 1:8. The absence of the signal characteristic of OCH₃ groups must have been reacted. At this point we are not sure about possible mechanism of this side reaction.

4.5. Starch Ester

The product obtained was rubber like solid with brown color and insoluble in D₂O, chloroform, dichloromethane, benzene, acetonitril, DMSO, and acetone. It starts to decompose at 277⁰C, but the melting point of the cassava starch is 170⁰C. Because of its insolubility it was not possible to characterize it using NMR.

In general, insolubility and higher melting point of the product indicates the starch may be self polymerized during the reaction process and poly-substitution is also expected.

5. Conclusions

Vernonia oil was extracted and refined and methyl vernolate (VOME) was successfully synthesized from it. Subsequently, VOME was reacted with starch and to give a product insoluble in most solvents. The product outcome from the reaction of VOME reacted with glucose (small monomer) was soluble in chloroform and could be analyzed using NMR. This analysis confirmed the condensation of starch/fatty acid and the ratio of epoxy and double bond remained the same. As a result the product obtained was biodegradable because both the epoxy and the double bonds can undergo hydrolysis.

Future work should include the analysis of insoluble product obtained from the reaction of cassava starch and the ester of fatty acid by different techniques such as solid NMR, IR and x-ray crystallography. These analysis techniques should help the elucidation of the structure of this product.

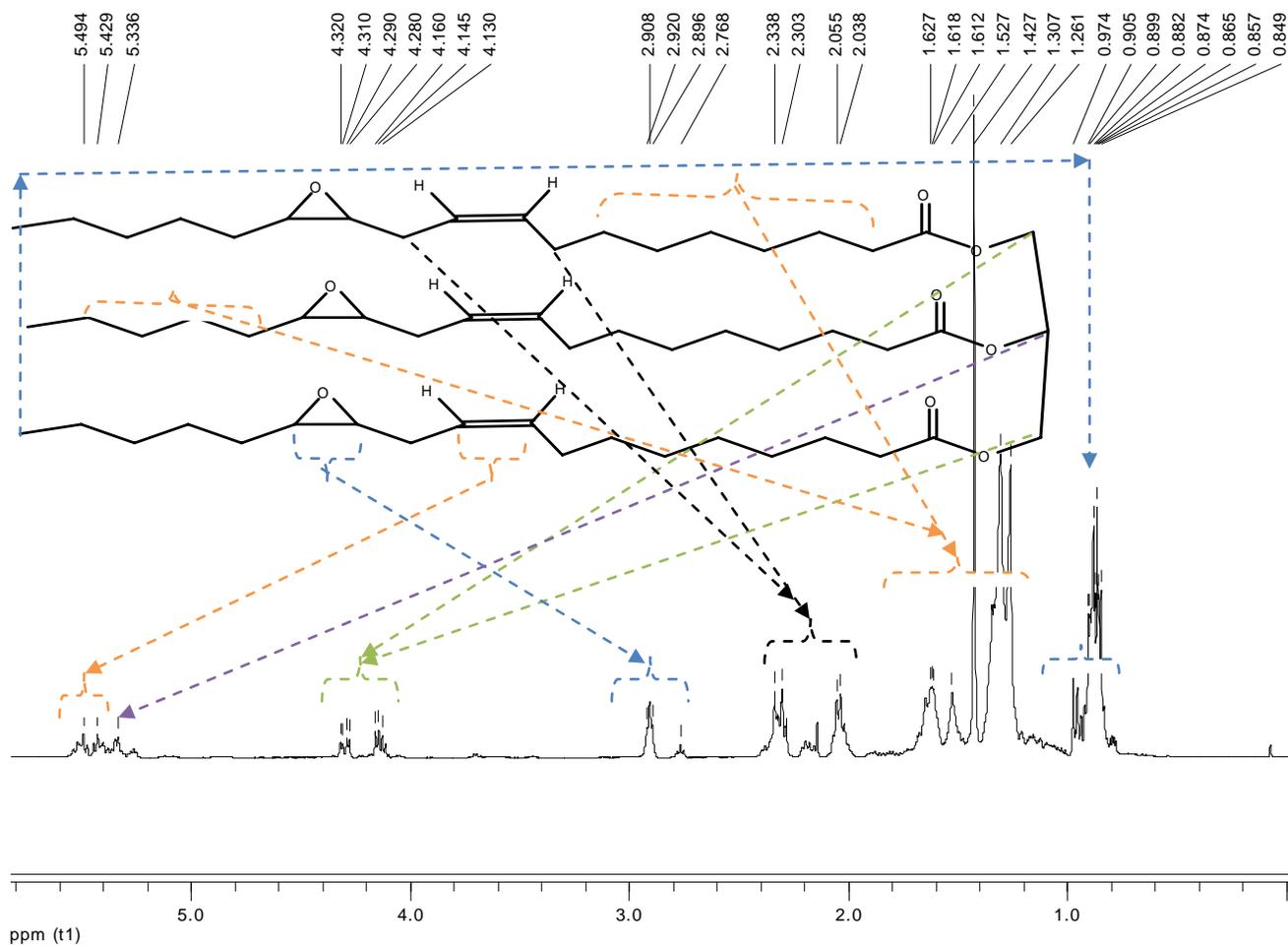
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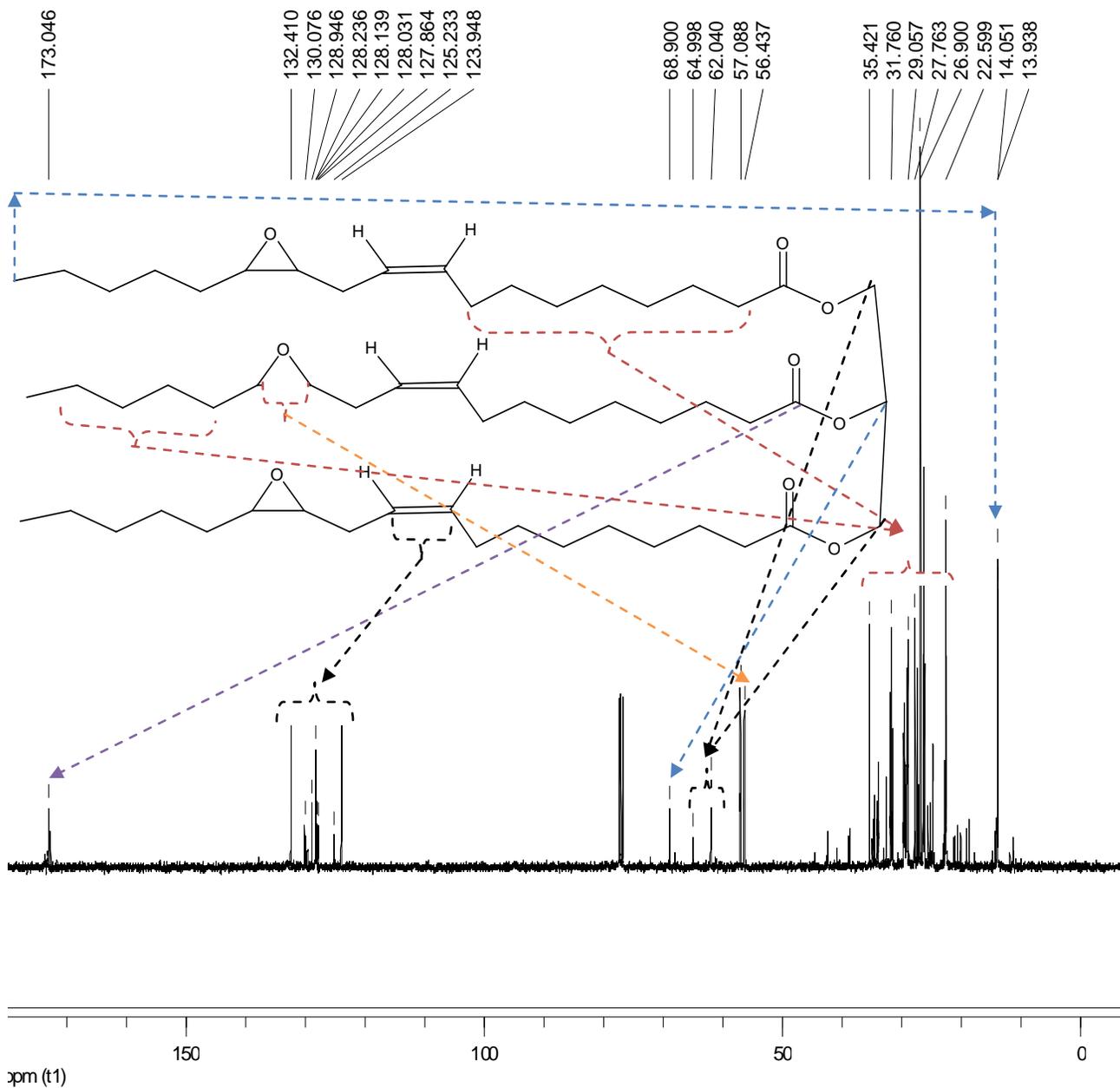
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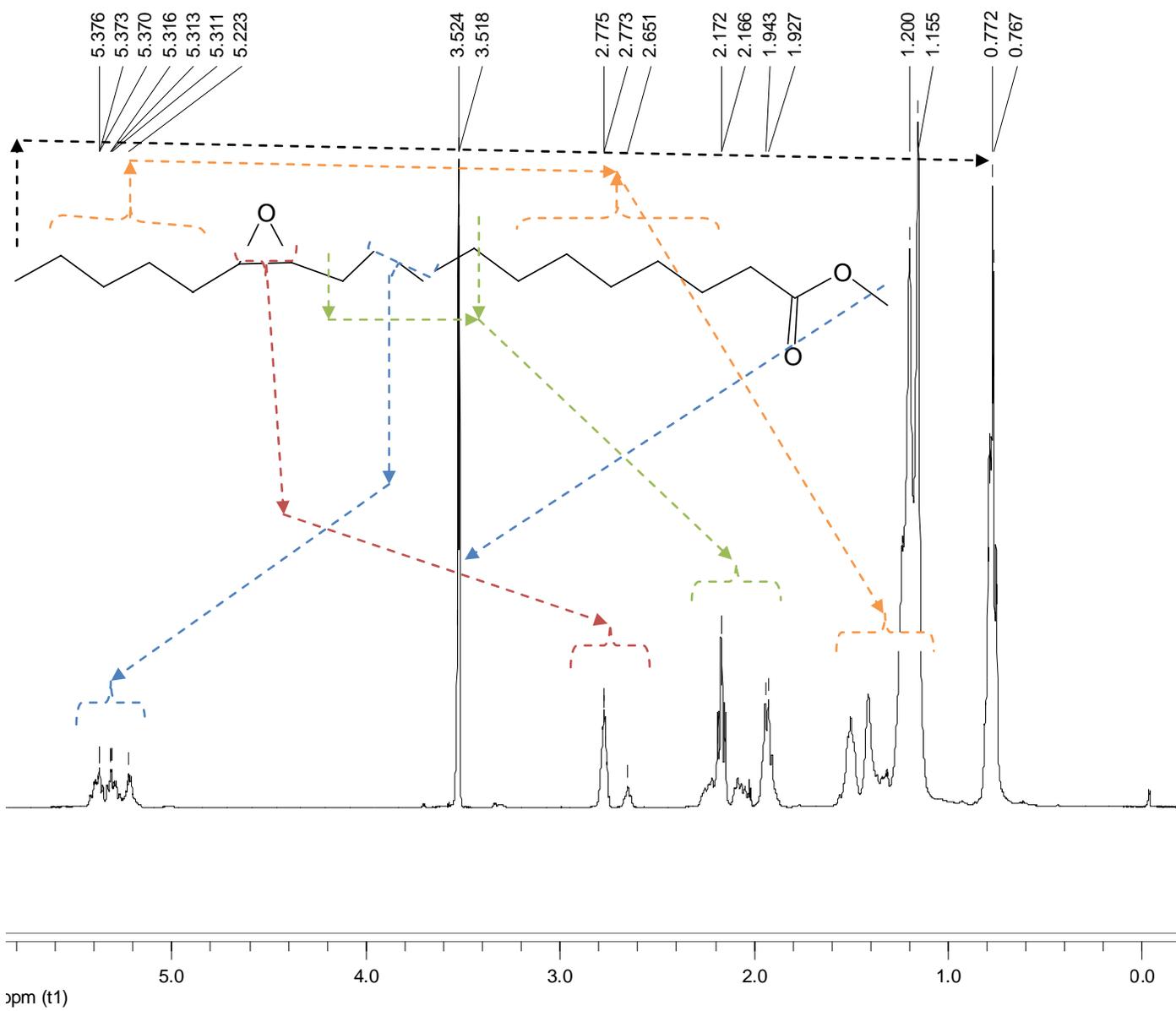
7. Appendices



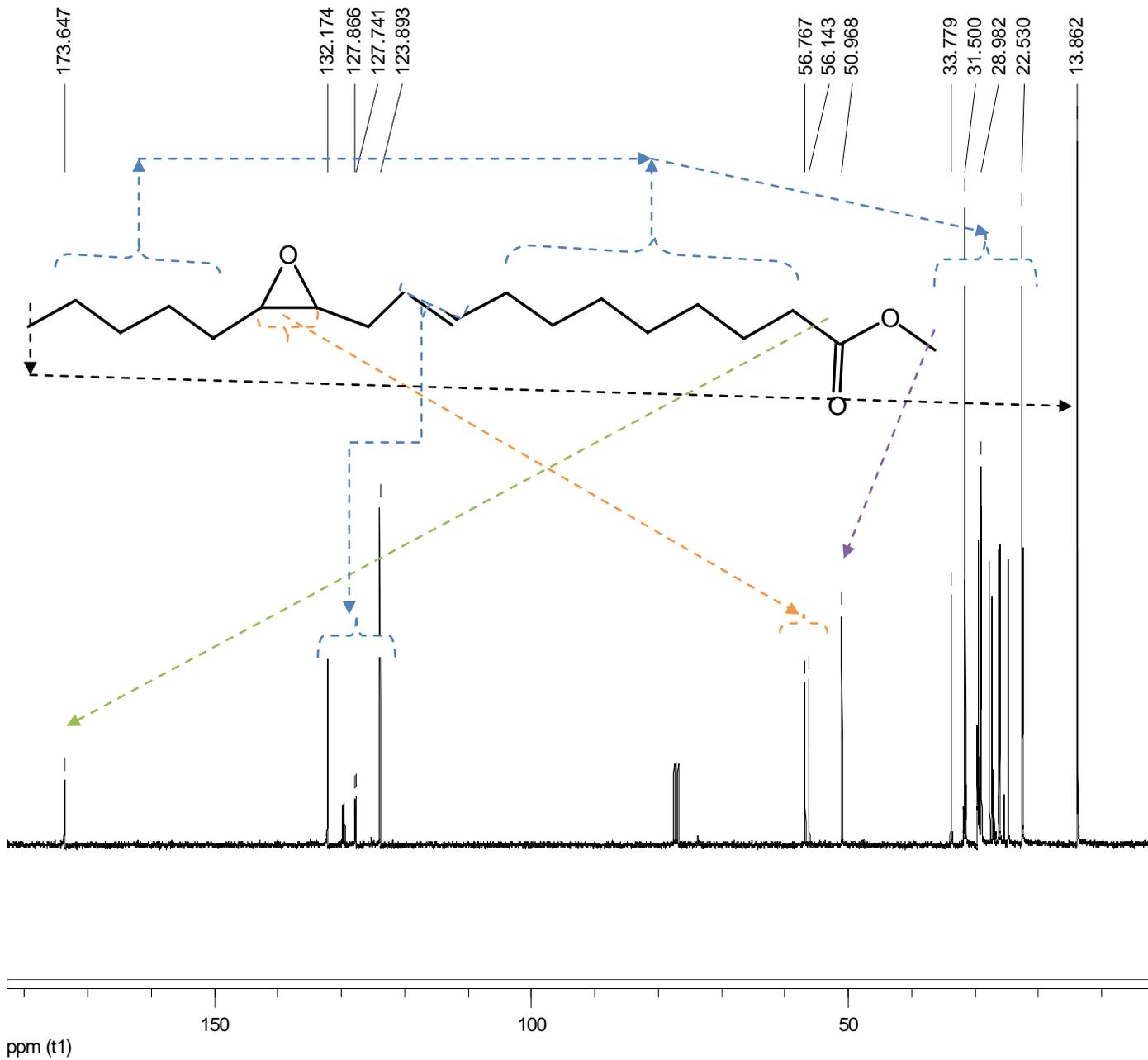
Appendix 1: ^1H NMR spectrum of the Vernonia oil.



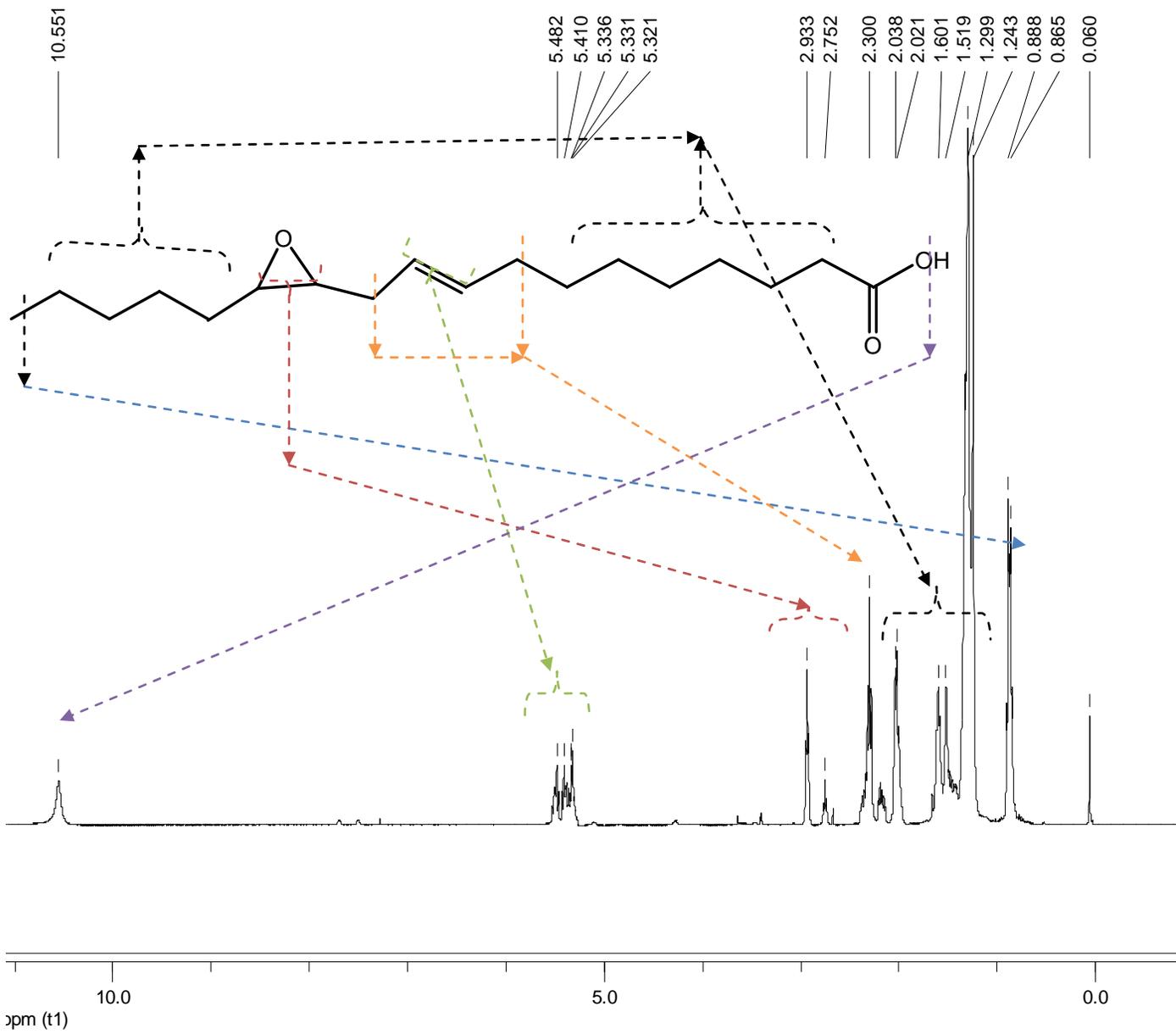
Appendix 2: ^{13}C NMR spectrum of the Vernonia oil.



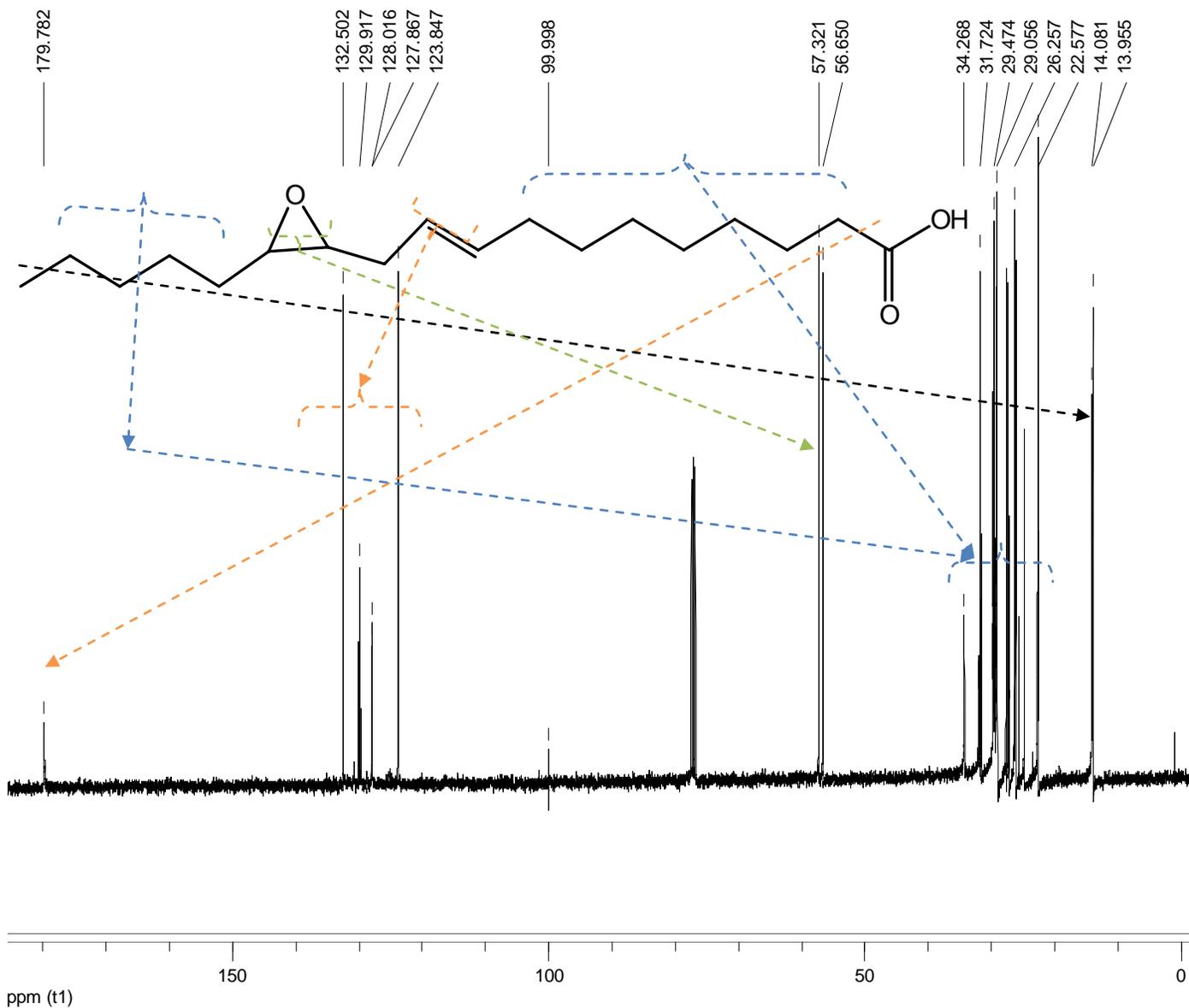
Appendix 3: ¹H NMR spectrum of VOME.



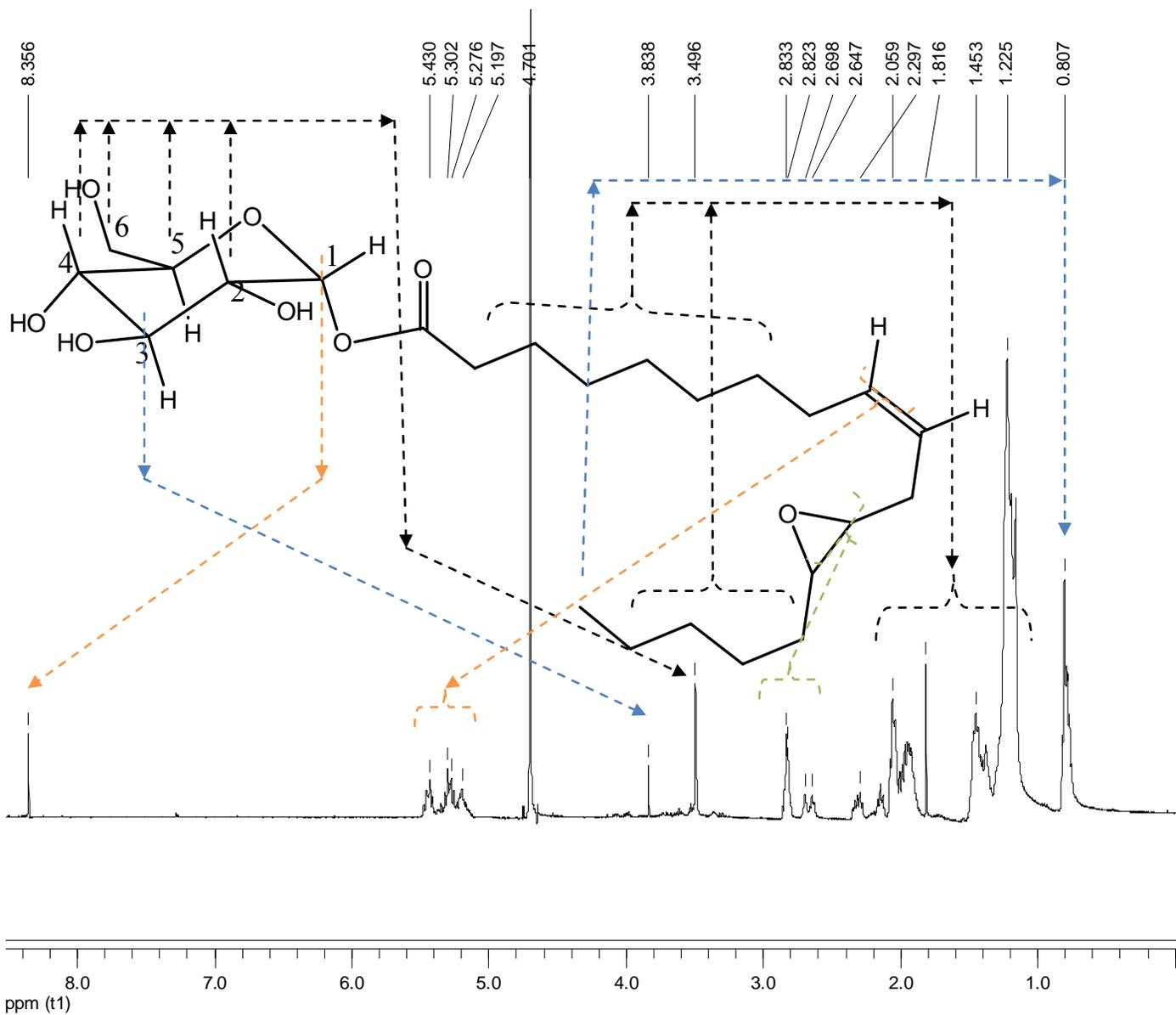
Appendix 4: ^{13}C NMR spectrum of VOME.



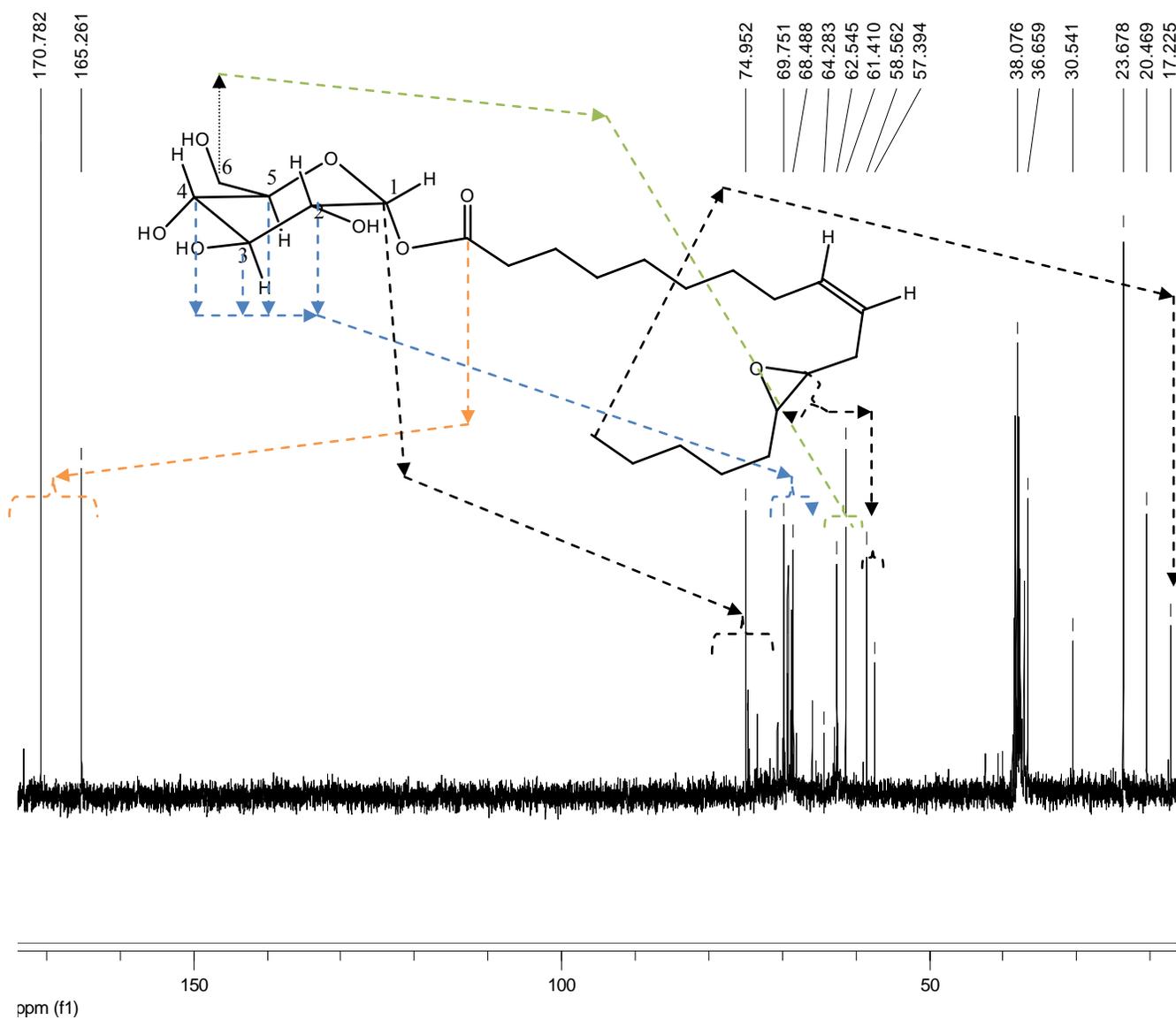
Appendix 5: ¹H NMR spectrum of Vernolic acid.



Appendix 6: ^{13}C NMR spectrum of Vernolic acid.



Appendix 7: ¹H NMR spectrum of the Glucose ester.



Appendix 8: ^{13}C NMR spectrum of the Glucose ester.