"THE EXTRACTION AND CHARACTERIZATION OF VERNONIA OIL FROM *VERNONIA GALAMENSIS* SEEDS AND ITS CONVERSION INTO DIBASIC ACIDS AND SOME ADHESIVE RESINS"

BY

REUBEN ROY KIMWOMI KIMWOMI

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This thesis is my original work and has not been presented for a degree in any other University.

Kimwomi R-R Kimwomi

REUBEN ROY KIMWOMI KIMWOMI

This thesis has been submitted for examination with our approval as University Supervisors.

Peter M Gitu

DR. P.M. GITU
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

Bhalendu Bhatt

DR. B.M. BHATT
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

DEDICATION

To my Mother, without whose dotting love I would not have made it out in life, and to my siblings.

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ABSTRACT

Vernonia galamensis is an oilseed crop with interesting chemical groups, having potential of being an important industrial crop, which would easily be grown in semi-arid areas. Extraction and subsequent oxidation was performed on the seeds.

Vernonia galamensis seeds contain lipase enzyme which was inactivated before extraction, by the use of three methods: passing steam through the seeds, oven heating and autoclaving. It was found that autoclaving was the better of the three. Solvent extraction yielded oil which was purified by the use of activated charcoal then hot-filtered using the aid of Celite (80-240 mesh). Degumming was done by stirring the oil with distilled water in the ratio of 21:1 (v/v) at 50°C for 1 hour. Centrifugation for about 3 hours was done and separation effected by decantation.

The extracted oil was shown to contain the epoxide ring by use of the picric acid method, and the percentage oxirane (3.954). Other physical properties include the saponification value (172.183); the unsaponifiable matter (4.172); the acid value (2.675); the iodine number (86.400); and the viscosity (109.974cps).

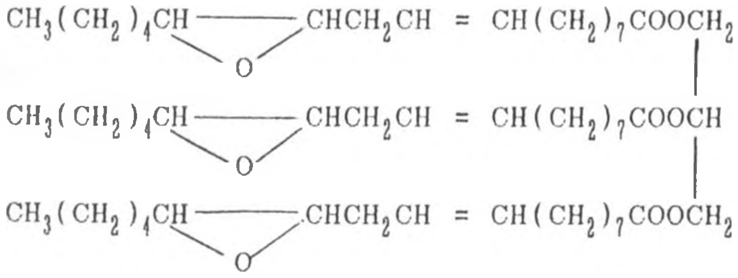
Refluxing the oil with acetic acid for 8 hours followed by hydrolysis with boiling alcoholic potassium hydroxide, subsequent acidification and extraction, yielded crystals of 12,13-dihydroxy-9-oleic acid.

$$CH_3(CH_2)_4CH(OH)CH(OH)CH_2CH = CH(CH_2)_7COOH$$

12,13-dihydroxyoctadec-9-enoic acid

The oxidation of this dihydroxyoleic acid using potassium permanganate yielded octanedioic acid and hexanoic acid. Another oxidation using lead tetraacetate produced an unsaturated aldehyde, which gave a deep orange 2,4-dinitrophenylhydrazone, while oxidation by the use of periodate-permanganate reagent yielded nonanedioic acid.

Vernonia oil is known to be a naturally occurring epoxidized oil existing as a triglyceride (trivernolin), possessing unsaturation.



trivernolin

GC analysis of the oil indicates that trivernolin is the major component triglyceride of vernonia oil from *Vernonia galamensis*, accounting for about 80% of the total oil content.

The reaction of the oil with diaminobutane yielded an interesting resin which had some rubbery-sticky characteristics. The possibility that this could form an adhesive formulation were considered. Phenol-formaldehyde resins were modified using vernonia oil. Instead of the brittle translucent plastic substance, a yellow-green oft plastic was obtained.

Vernonia oil is apparently rare in nature by bearing natural epoxy groups in high concentration, in addition to unsaturation. If the crop is commercially cultivated it could significantly boost the library of important chemicals, and provide a new source of compounds. As a renewable resource, vernonia oil appears to have an important future.

CHAPTER 1

1.0.0 INTRODUCTION

Vernonia galamensis (cass.) less (syn. *Vernonia pauciflora*) is a new potential oil crop. Its seeds contain an epoxidized oil, 80% of which is a triglyceride of vernolic acid (vernolin). The fact that this naturally occurring oil contains an epoxide ring, makes it a primary candidate for application in the coatings and plastic industries[1]. The oil could possibly be used in the preparation of reactive diluents for high solids coating formulations[2]. This would lead to the reduction in the use of non-reactive diluents in the coatings formulations for air pollution control.

1.0.1 DESCRIPTION OF THE PLANT

The genus *Vernonia* belongs to the family *Compositae*, which are annual or perennial herbs, small to large shrubs, woody scramblers and climbers, or rarely small trees[3]. The plant, a native of Eastern and Central Africa, is a relative of the thistle. It is an annual herbaceous plant varying from small ephemerals 20 cm tall with a single flower head to robust, rather diffusely branching somewhat shrubby plants, to 5 m tall with many flower heads. It never branches from the base, but flowers .after the first flower head is formed. The flowers are lavender and set one-inch clumps of brown seeds, leaves are alternate sessile membraneous, 0.6 cm to 5.0 cm wide up to 25 cm long. They are acuminate at the tip but cuneate at the base. The margins are toothed and the surface puberulous to pilose[2]

1.0.2 VERNONIA GALAMENSIS: DISTRIBUTION

According to Gilbert[1], *V. galamensis* includes six subspecies one of which includes four varieties:

1. ssp. *galamensis*
   (a) var. *galamensis*
   (b) var. *petitiana* (A rich) M.Gilbert
   (c) var. *australis* M. Gilbert
   (d) var. *ethiopica* M. Gilbert
2. ssp. *nairobensis* M. Gilbert
3. ssp. *lushotoensis* M. Gilbert
4. ssp. *mutomoensis* M. Gilbert
5. ssp. *afromontana* (R.E. Fries) M.Gilbert
6. ssp. *gibbosa* M. Gilbert

As far as distribution is concerned, *V. galamesis* segregates occur in areas with annual rainfall as low as 50 cm, thus it grows well in both arid and semi-arid areas of the tropics and subtropics, on land that is practically unsuitable for food crops. It can also grow in rich forested areas with annual rainfall of 185 cm. In Kenya, *V. galamensis* is found growing as weed in

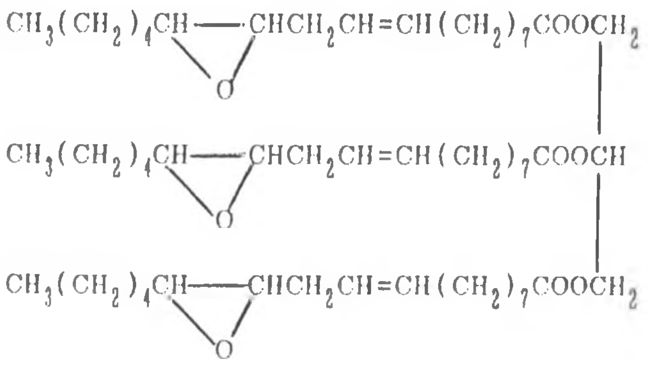
Nairobi, Central, Rift Valley and Eastern Provinces. It is very likely that this species grows in all provinces of Kenya.

1.1.0 BACKGROUND

Studies done on *Vernonia anthelmintica*, which belongs to *Vernonia* section *Stengelia*, vis à vis *V. galamensis*, show that even the unimproved varieties of the latter, contain about 30% more vernolic acid than the best improved varieties of the former[5].

1.1.1 VERNONIA OIL

Vernonia oil is a naturally occurring epoxidized oil existing as a triglyceride (trivernolin) of vernolic acid, containing three epoxy rings, one on each vernolic residue (See structure below).



Trivernolin

It also contains three carbon-carbon double bonds, one on each vernolic acid residue. This oil contains 80% trivernolin and is a transparent, clear, homogeneous liquid at room temperature, with excellent solubility in many organic solvents and diluents used in paints. It also has low viscocity and low melting point, an aspect of importance in all reactive diluents for coating applications[6]. The molecular structure consists of three equal vernolic acid residues, unlike other vegetable oils with differing fatty residues.

The epoxy oil from *Vernonia galamensis* seed has properties such as oxirane content (4.1%), viscosity (110 cps) and molecular weight (917) that relate to commercial epoxidized triglycerides. The *V. galamensis* seeds yield 40-42% oil containing 72-78 % vernolic (*cis*-12,13-epoxy-*cis*-9-octadecenoic acid), thus making it an excellent source of naturally epoxidized oil. The presence of vernolic (76%), oleic (4%) and linoleic (13%) acid levels in *V. galamensis* oil represent additional epoxidizable unsaturation (110%) monoene equivalents so that fully epoxidized vernonia oil could have an oxirane value of near 10%[7]. Potential demand for vernonia oil as a reactive diluent for alkyd-resin paints, the kind of oil based paint used on buildings, has been estimated at 40 million gallons, based on the use of one pint of vernonia oil per gallon of paint, according to John C. Graham, Director of the Coatings Research Institute at Michigan University. He says that formulating alkyd-resin paint with vernonia oil could reduce emissions involved in photochemical pollution by as much as 160 million pounds per year[1]. Several initial attempts for utilization of vernonia oil in different areas have been reported: interpenetrating polymer networks based on the oil, good coatings obtained directly from oil, further epoxidization and oxidation to prepare dibasic acids[5].

1.2.0 LITERATURE REVIEW

R.E. Perdue[2] did a study on *Vernonia galamensis* seed from an arid area of Eastern Ethiopia in 1964 and found out that the plant contained 41.9% oil with 72.6% vernolic acid. In December 1966, preliminary research was done on *Vernonia galamensis* species from the Kenya Highlands[2]. It was found that the seed contained 29.9% oil and vernolic acid yield was up to 78.2% of the oil. In 1975, the seeds were grown in Kericho, Kenya and found to do quite well with no evidence of insect disease or other problems[4]. In India, vernonia oil has been used as an anthelmentica for hook worm[3] and as a remedy for leucodema[7]. Some of the species of vernonia have been shown to contain active compounds which possess cytotoxic and antitumour activity attributed to sesquiterpene lactones,[8,9,10] isolated from more than 70 species of vernonia during chemical evaluation of the genus. Vernolepin, isolated from the leaves of *V. hymenolepsis*, was found to be cytotoxic to tKB cells in vitro[11]. The role such toxic materials might play in feeding defatted seed meal to animals is unknown.

Analysis of defatted flakes and oil have shown that there is high crude protein levels(42.5%) accompanied by high levels of crude fibre (10.9%)[12]. *V. anthelmintica* seed meal amino acid analysis indicated that methionine and lysine were limiting factors for its use as the only source of protein for animal feeding. However, *V. galamensis* meal has higher lysine, methionine and phenylalanine levels. Essential amino acids are comparable to those in defatted soy meal[13].

1.3.0 VERNONIA OIL AND ITS POTENTIAL USES

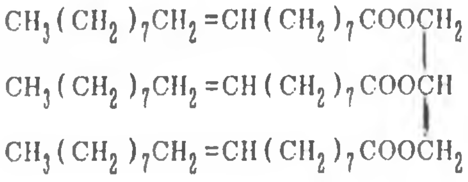
1.3.0.1 POLYMERS AND REACTIVE DILUENTS

Vernonia oil has been utilised in the making of interpenetrating polymer networks (IPNs), a combination of two polymers in network, at least one of which is synthesised. This is formed when a network of one polymer is swollen with a monomer[14]. Simultaneous interpenetrating networks (SIN) have also been prepared using this oil. SINs are formed when both polymerizations are carried out simultaneously[15]. For instance, Afolabi *et.al.*[16] have synthesised a toughened elastomer from *V. galamensis* seed oil by reacting it with suberic acid obtained from the oil, by cross-linking with styrene prepared in situ. As a reactive diluent, vernonia oil can be used to replace many conventional solvents which produce volatile organic compounds (VOC) emissions in line with the international environmental awareness which dictates that there be a reduction of VOC emission of paints and varnishes.

1.3.0.2 VERNONIA OIL AS A COATING OIL

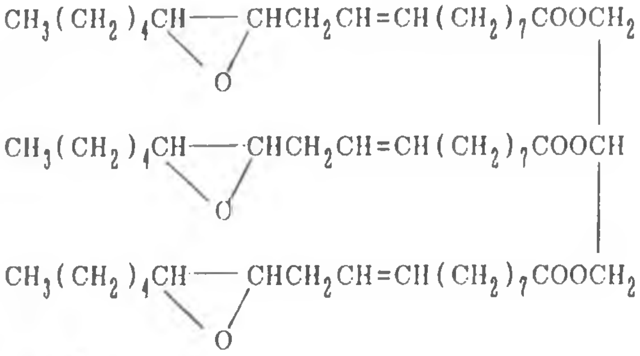
Vernonia oil can be used as a drying oil. Drying oils are relatively unsaturated being composed of triglycerides constructed from unsaturated fatty acids, and have an ease of auto-oxidation and polymerization to form hard dry films on exposure to air. Good drying oils have several non-conjugated double bonds per molecule. When compared to triolein and soybean oil that is epoxidised, in the presence of 0.5% cobalt drier under baking conditions at 150°C for one hour it was found that triolein and vernonia oil formed good coatings, but vernonia oil dried faster than triolein. On the other hand, soybean oil did not form coatings even at 185 °C for several hours. For comparison the molecular structures of the oils are given below[6].

TRIOLEIN

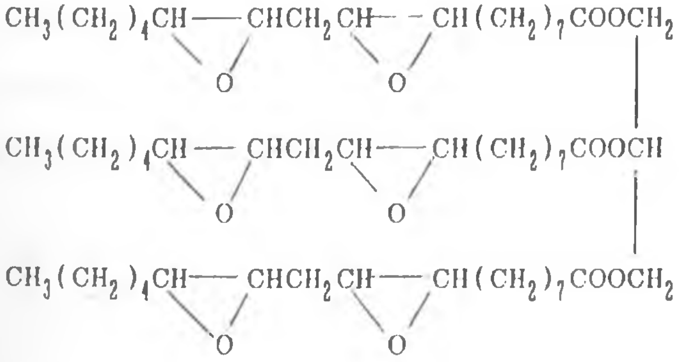


$$\begin{array}{l} CH_3(CH_2)_7CH_2=CH(CH_2)_7COOCH_2 \\ CH_3(CH_2)_7CH_2=CH(CH_2)_7COOCH \\ CH_3(CH_2)_7CH_2=CH(CH_2)_7COOCH_2 \end{array}$$

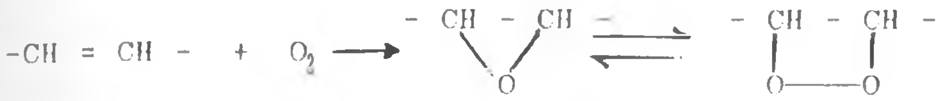
VERNONIA OIL



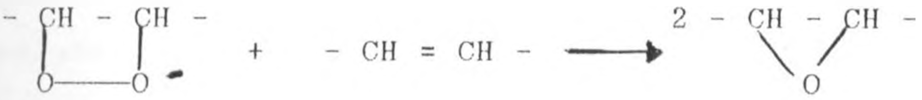
EPOXIDISED SOYBEAN OIL



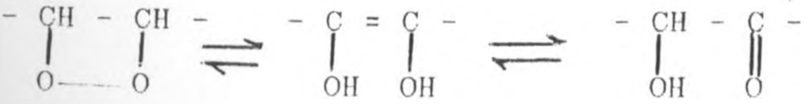
The epoxy rings of vernonia oil seem to activate the oxidation of the methylene group between the epoxy ring and the carbon-carbon double bond, facilitating a faster drying. The following schemes of reaction have been mentioned for the drying process by the atmospheric oxidation of the oil[17].



Two molecules of an epoxy compound might result when the peroxide interacts with a further unsaturated radical.



The fact that two atoms of oxygen are seen to be taken up renders this explanation less credibility and another view was developed that the organic peroxides undergo rearrangement into isomeric forms:



$$-\underset{\substack{|\\O}}{CH}-\underset{\substack{|\\O}}{CH}- \rightleftharpoons -\underset{\substack{|\\OH}}{C}=\underset{\substack{|\\OH}}{C}- \rightleftharpoons -\underset{\substack{|\\OH}}{CH}-\underset{\substack{\|\\O}}{C}-$$

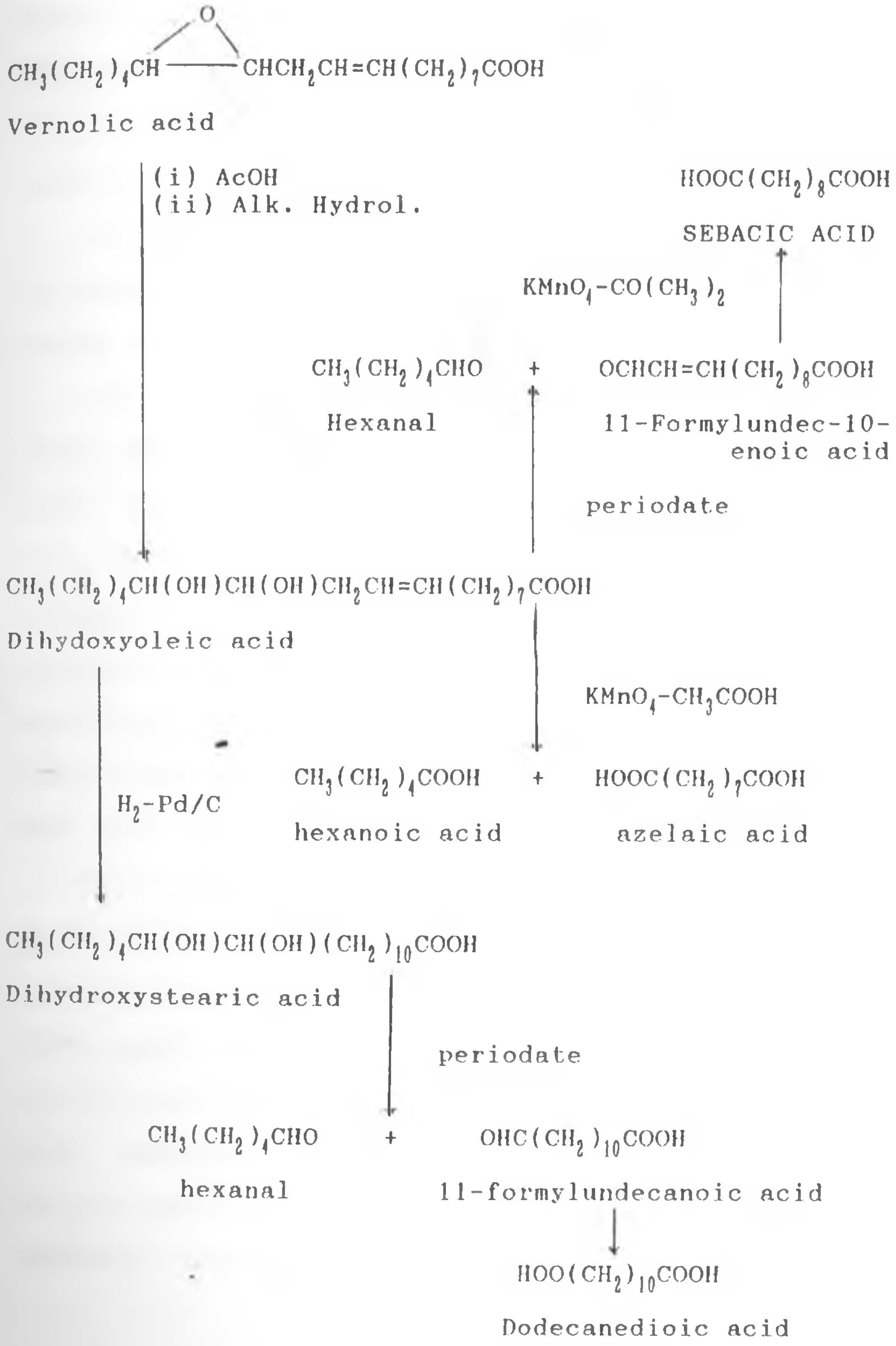
1.3.0.3 VERNONIA OIL AS A MODIFIER

In the modification of the epoxy resins, vernonia oil helps improve fracture toughness and since the oil is resistant to water, it injects the property of low water absorption to the epoxy resin. The commercial epoxy resin modified in this manner by introduction of vernonia oil will result in price reduction[6]. The oil has been used in the modification of polyvinylchloride and epoxy resins. It has also been reported that dibasic acids have been prepared by the oxidation of vernonia oil[18,19].

1.3.0.4 DIBASIC ACIDS FROM THE OIL

Aliphatic dibasic acids are established industrial raw materials that are used as plasticizers, and in the manufacture of engineering fibres, synthetic fabrics and impact resistant elastomers. Most of these are available from synthesis utilizing petrochemical feedstock[20,21]. Gunstone has shown that vernolic acid could undergo the following reactions to form dibasic acids as shown in SCHEME 1 below[22].

SCHEME 1 DIBASIC ACIDS FROM VERNOLIC ACID



Most dibasic acids have been found to be present in natural plants and the following acids have been successfully produced :

(i) Oxalic acid, HOOCCOOH, found in plants such as sweet potatoes, cabbages, grapes, and tomatoes[23].

(ii) Adipic acid, $HOOC(CH_2)_4COOH$ is one of the compounds formed when many unsaturated fats or fatty acids are oxidised[24].

(iii) Suberic acid, $HOOC(CH_2)_6COOH$ is obtained in small amounts by the oxidation of castor oil with nitric acid. Suberic acid has also been prepared from vernonia oil. This acid is used in the manufacture of alkyd resins. The use of diamines yields polyamides which on polymerization yields a Nylon (Type 8) which finds application in coatings, textile finishes, and adhesives where toughness, flexibility and hydrocarbon resistance are required[25].

(iv) Azelaic acid was first prepared by Ganttner and Hell in 1881[26]. Saytzeff in 1885[27] obtained suberic and azelaic acids from castor oil. Oxidative fission of oleic acid with potassium permanganate can be carried out to give relatively good yields of azelaic acid but the reaction cannot be controlled to produce stoichiometric quantities of the primary fission products indicated by the following equation:

$CH_3(CH_2)_7CH=CH(CH_2)_7COOH \longrightarrow$
oleic acid

$CH_3(CH_2)_7COOH$ + $HOOC(CH_2)_7COOH$
nonanoic acid azelaic acid

Both of the primary fission products undergo further oxidation to produce shorter chain acids prior to complete oxidation of the oil. Azelaic acid has been prepared by oxidation using KIO_4, $KMnO_4$ from cameline oil as well as oil from *V.anthelmintica*[22].

1.3.0.5 DIBASIC ACIDS: PROPERTIES

The dicarboxylic acids, like all polyfunctional compounds, have certain characteristic behaviour depending on the relative positions of the functional groups. Being an electron withdrawing group, the presence of one carboxyl close to another increases the ease of ionization of the first hydrogen ion. This effect decreases as the carbonyl groups become more separated. Solubilities in water exhibit alternation, the acids with an odd number of carbon atoms being more soluble than the adjacent even numbered acids. Melting points of dibasic acid also exhibit alternation similar to that observed with monobasic acids. Within the series of alternate pairs, the melting point decreases with increasing methylene groups such that oxalic acid $(COOH)_2$ melts at 189.5°C while dodecanedioic acid,

$HOOC(CH_2)_{10}COOH$ melts at 126°C. From adipic acid, the higher acids having the carboxyl groups more widely separated do not give cyclic anhydrides as the lower ones. On heating with dehydrating agents they give linear polymeric anhydrides[28].

TABLE 1.1 PROPERTIES OF DIBASIC ACIDS OBTAINED BY OXIDATION OF UNSATURATED FATTY ACIDS

NAME OF ACID	FORMULA	M.P (°C)	B.P. (°C)
OXALIC	$HOOCCOOH$	189.5	-
MALONIC	$HOOCCH_2COOH$	135.6	-
SUCCINIC	$HOOC(CH_2)_2COOH$	185.0	235.0
GLUTARIC	$HOOC(CH_2)_3COOH$	97.5	304.0
ADIPIC	$HOOC(CH_2)_4COOH$	153.0	265.0
PIMELIC	$HOOC(CH_2)_5COOH$	105.5	272.0
SUBERIC	$HOOC(CH_2)_6COOH$	142.0	300.0
AZELAIC	$HOOC(CH_2)_7COOH$	108.0	360.0
SEBACIC	$HOOC(CH_2)_8COOH$	134.0	395.0
HENDECANEDIOIC	$HOOC(CH_2)_9COOH$	110.0	-
DODECANEDIOIC	$HOOC(CH_2)_{10}COOH$	126.0	-
BRASSYL	$HOOC(CH_2)_{11}COOH$	112.0	-

Except for oxalic and malonic acids, these dibasic acids are readily distillable without decomposition.

1.3.1 OXIDATION OF UNSATURATED ACIDS

When unsaturated fatty acids are oxidised, various products ensue where the number of double bonds determines products. When linoleic acid is oxidized in a quantitative manner, with the subsequent degradation of the primary products, the following acids would form: caproic acid, malonic acid, and azelaic acid as shown below:

$$\begin{array}{c} CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH \\ \Big\downarrow \text{ oxidation} \\ CH_3(CH_2)_4COOH \;+\; HOOCCH_2COOH \;+\; HOOC(CH_2)_7COOH \end{array}$$

Hilditch[17] has shown that prolonged alkaline permanganate oxidation of unsaturated acids produces a series of acids some containing one less carbon atom than the expected. For example, in the oxidation of octadecenoic acid, valeric acid, $CH_3(CH_2)_3COOH$ is seen instead of the expected caproic acid and oxalic acid appears where malonic acid is expected.

1.4.0 PHYSICAL CHEMICAL PROPERTIES DETERMINATION

1.4.0.1 SEED TREATMENT

In the extraction of the oil it has been observed that lipolytic activities give rise to high free fatty acid (FFA) levels. Thus precautions should be taken to avoid this situation by tempering the seed. The recommended method involves autoclaving the seeds and then adding a moisture content of about 15%[1].

1.4.0.2 OIL EXTRACTION

Oils may be extracted by cold pressing in hydraulic presses or in continuous expellers. This method has been used in the extraction of olive, cotton seed, and peanut oils. The same method is being adopted for the extraction of vernonia seed oil. Another method of oil extraction is hot pressing but this may give a large quantity of undesirable constituents though it could result in a high yield. Solvent extraction is by far the most efficient means of oil extraction and it is the method to be adopted in this work. The extracts are solvent stripped using a rotary evaporator at 60°C and then desolvented to the atmosphere in a hood over 48 hours. The crude oil is then refined with activated charcoal at levels of 2 to 8 % by weight. This is done by mixing the oil with the charcoal and stirring at 60°C for 1 hour followed by filtration.

The decolorised oil is then degummed by stirring with distilled water at the ratio 21:1 at 50°C for 1 hour followed by centrifugation for 3 hours. This degummed oil is then alkali refined at 40°C by adding 2N NaOH in the ratio 31:1 with stirring for 30 minutes.

1.4.0.3 ACID VALUE

Fatty acids in oils and fats occur either as free fatty acids or as esters, when they are bound to the triglycerol molecule. The acid value therefore is an indication of the amount of free fatty acids, as percentage oleic, percentage palmitic or percentage lauric acid in the sample, depending on the kind of oil or fat being handled. Determination can be carried out at the same time as that of saponification value. It can be done by boiling the neutral alcohol suspension of the fat or oil until all is liquid. Adding alcoholic potash by titration in the presence of phenolphthalein until an almost pink colour is produced, then proceeding to add the remainder of 25 CM^3 of alkali from the burette[17].

1.4.0.4 CONTENT OF UNSAPONIFIABLE MATTER

Unsaponifiable matter give the amount of neutral (non-acidic) material, substances that are insoluble in water and present either free or combined with fatty acids in the original material. In situations where large amounts of higher fatty alcohols or sterols are present as esters (e.g. sperm oil, bees wax, carnauba and other waxes or wool greases), then combined alcohols in question are registered as unsaponifiable matter in this estimation. This property in addition gives a measure of any free sterols or other water-insoluble alcohol, hydrocarbons or other neutral organic compounds being examined[17].

1.4.0.5 THE IODINE VALUE

The iodine value is a reflection of the degree of unsaturation in a sample. Many methods for determining iodine value have been devised. The results are commonly expressed as percent iodine absorbed, i.e., grams of iodine per 100 grams of sample, whether or not the halogen used is actually iodine. The widely used methods are the Wijs' and Hanus'. The difference is very small with fats of low iodine value, but often is 2% or more with oils having iodine of 100 or more with conjugated oils, on the other hand, Hanus' method gives considerably higher values, but more variable ones, than Wijs' method. Wijs' values obtained with non-conjugated oils are believed to be very close to those corresponding to actual unsaturation[17].

1.4.0.6 PREPARATION OF STARCH SOLUTION

Starch is required for the determination of iodine value. Solution starch is made by filtering a dilute solution of starch so as to remove cellulose fragments, then precipitating the starch solution by alcohol. The white powder so obtained is collected and dried, and when boiling with water readily dissolves, yielding a liquid which gives a clear deep blue colour with a drop of iodine solution. On account of this superior sensitiveness, soluble starch should always be employed in preference to the ordinary kind. The solution should be dilute, since a very small quantity of starch is required to react with iodine.

1.4.0.7 PREPARATION OF SODIUM THIOSULPHATE SOLUTION

Iodine and sodium thiosulphate interact to produce sodium iodide and sodium tetrathionate, and any amount of iodine present in solution, whether liberated from an iodide by an oxidizing agent or not, can be estimated accurately by this reaction because of the delicate test for free iodine with starch. A tenth of a mole solution of sodium thiosulphate reagent contains 24.82 g per litre. The salt can be obtained chemically pure, but its solution is decomposed by carbon dioxide dissolved in water, liberating thiosulphuric acid from which sulphur is slowly decomposed. It is well, therefore, to prepare a solution of strength slightly more than a tenth of a mole, and to allow it to stand for a few days before standardizing to give time for its interaction with dissolved carbon dioxide.

1.1.0.8 PREPARATION OF POTASSIUM DICHROMATE SOLUTION

In iodine value determination it is required that sodium thiosulphate standard solution be used. This means that standardization of the solution be made by standard potassium dichromate, which is an important reagent in volumetric analysis because of its oxidizing property. Oxidation of the dichromate involves reduction of chromium from the state represented by CrO_3 to that represented by Cr_2O_3.

1.1.0.9 SAPONIFICATION VALUE

This is the number of milligrams of KOH required to hydrolyse 1 g of a fatty material[29]. Data expressed in this form approach simple numerical indices which are extremely useful to analysts who are largely occupied with the assessment of fats in foods or other goods. Sometimes saponification value is replaced by saponification equivalent. Saponification equivalent is the amount of fat or wax saponified by one gram-equivalent of KOH, and is therefore a mean gram-equivalent of the mixture of triglycerides of wax esters present in a material being examined, assuming that the substance consists exclusively of neutral esters of these types. Saponification equivalent which is related to the saponification value by the formula[17]:

$$\text{saponification equivalent} = \frac{56\ 100}{\text{saponification value}}$$

affords a fairly clear indication of the mean molecular magnitude of an ester (triglyceride or otherwise) which is present.

1.4.1 GAS CHROMATOGRAPHY

Chromatography is a technique for separating a mixture into its individual components. Chromatographic separations are carried out by indigenous mechanical manipulations involving a few of the general physical properties of molecules. The major properties are tendency for a molecule to

(i) dissolve in a liquid (solubility),

(ii) attach itself to a finely divided solid (adsorption) and

(iii) enter the vapour state or evaporate (volatility).

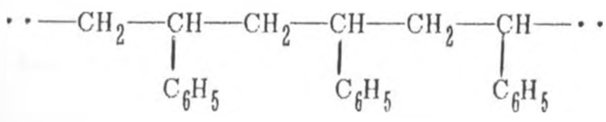
Mixtures of substances to be separated are placed in a dynamic or moving environmental situation where they can exhibit two of these properties. In gas chromatography, separation depends upon the partition (or distribution) of compounds between a moving phase (the carrier gas) and the stationary phase a non-volatile liquid adsorbed on an inert support such as kieselguhr. As the moving phase flows through the column packed with a stationary phase, the sample also travels along and is separated into its components by virtue of differences in the first place, of solubility of these components in the stationary phase and secondly, of volatility[30].

1.5.0 MACROMOLECULES

Synthetic linear organic polymers are said to be the simplest of all macromolecules. These consist of long unbranched chains of small identical sub-units, or two to three different kinds of subunits. Examples are polymethylene prepared from diazomethane[31].

$$\cdots - CH_2 - CH_2 - CH_2 - CH_2 - \cdots$$

Polystyrene, prepared from styrene



$$\cdots - CH_2 - \underset{C_6H_5}{\underset{|}{CH}} - CH_2 - \underset{C_6H_5}{\underset{|}{CH}} - CH_2 - \underset{C_6H_5}{\underset{|}{CH}} - \cdots$$

Polyesters prepared from the condensation of

$HO-(CH_2)_x-OH$ and $HOOC-(CH_2)_y-COOH$

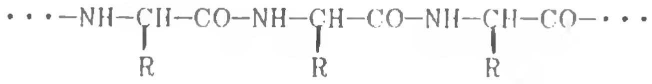
$$\cdots - O - (CH_2)_x - O - CO - (CH_2)_y - CO - O - (CH_2)_x - O - CO - (CH_2)_y - CO - \cdots$$

Polyamides prepared by the condensation of

$H_2N—(CH_2)_x—NH_2$ with $HOOC—(CH_2)_y—COOH$

$\cdots$–$HN-(CH_2)_x-NH-CO-(CH_2)_y-CO-HN-(CH_2)_x-NH-CO-(CH_2)_y-CO-$..

And polypeptides prepared from the corresponding N-carboxy anhydrides



$$\cdots-NH-\underset{R}{\underset{|}{C}}H-CO-NH-\underset{R}{\underset{|}{C}}H-CO-NH-\underset{R}{\underset{|}{C}}H-CO-\cdots$$

As was shown by Afolabi et.al.,[16] the epoxy group in the oil is a reactive centre in its reaction with suberic acid. In this work, there will be an attempt to react this group with diamines and see whether a polymer would ensue.

1.6.0 ADHESIVES

An adhesive is a material employed between surfaces to make them stick together or cling. Thus an adhesive bonds two surfaces by adhering strongly to the surfaces of both. In most cases the formation of an effective adhesive bond depends upon conversion of the adhesive layer itself to a semi-solid jelly or hard film, which of course, have both good cohesive strength and firm attachment to the materials being bonded together.

Adhesives of the type of the water soluble glues have been in use for a very long time. Recent developments in other adhesives have led to a marked expansion in the use of adhesive in industry. Heat sealing adhesives, applied in thin layers on paper, cellophane, etc., made rapid strides in the packaging industry, just as chemically reactive adhesives have replaced soldering, welding, rivetting etc., in the fabrication of many products. Pressure sensitive ones are not to be left out as they compete to expedite many manufacturing operations[32].

1.6.1 FACTORS AFFECTING ADHESION

Practical observation, together with the fundamental studies of McBain J.W. et.al.[33], indicate that effective action depends on the following factors:

(a) affinity of the adhesive for the material to be bonded;

(b) the tendency of the adhesive to wet the material to be bonded;

(c) the tendency of the adhesive to penetrate the surface of the material to be bonded;

(d) the strength and continuity of the adhesive material in itself; and

(e) the thickness and flexibility of the adhesive layer.

In the case of adhesives containing ingredients having reactive groups, the affinity of the adhesive for the material to be bonded may be very important. In fact, chemical reactions leading to a very strong bond may result. Adhesives having a good internal strength and continuity of their own are usually desired, with the most favoured being those having strength at least equal to that of the materials to be bonded. There are two obvious conditions necessary for the successful functioning of an adhesive :

(a) contact in molecular dimensions

(b) a lower adhesive-solid interfacial boundary energy than the sum of the surface energies of solid and adhesive.

(A) The requisite for proper contact is flow of the adhesive which can be induced by solvent, heat, or pressure.

(B) Quantitative data on boundary energy is lacking but known values give immense information on the type of adhesive. Compatible material tend to be soluble in one another, and tend to give the highest adhesions of one another. Such materials have low interfacial surface energies. When the solvent for the adhesive is a solvent for the material being bonded, the interface can disappear altogether. The interfacial energy is dissipated as heat of solution of solid-solid in case of this type .of adhesives. Such adhesives are quite tenacious. Another tenacious bond is formed by introducing a polymerizable material between pieces of solid polymer, in which case the bond can result from a chemical union of polymerizing adhesive and solid polymer[34]. Polar adhesives bond best to polar materials and nonpolar adhesives to nonpolar materials. In forming an adhesive bond to a flexible, porous material such as cardboard, it is often desirable to obtain contact with a large surface area of the weak material. Absorption of the adhesive by the porous material, or even partial impregnation of the latter, not only gives this enlarged surface area but may also strengthen the material. Another example of the importance of mechanical factors involves the bonding of two dissimilar, rigid materials of different coefficients of expansion. The most suitable adhesive for bonding is one which can distribute the stresses set up during temperature variation. Such an adhesive may have elastic properties.

The various types of adhesives, classified on the basis of how the forces mentioned above are brought into operation, are discussed below under the following headings:

(1) solvent-responsive or solvent activated

(2) heat-responsive or heat sealing

(3) pressure-responsive or pressure sensitive, and

(4) chemically active.

1.6.2 SOLVENT-RESPONSIVE

Solvent-responsive or solvent-activated adhesives are effected by means of a volatile liquid carrier. Such adhesives are used as solutions, dispersions, pastes and gels (the last are liquified by heating when applied). Of all adhesive compositions these have been in use for the longest time, are now the most widely varied in range of useful constituents, and are still the most prevalent in use. These include protein glues, vegetable gums and starches, (reported to have been in use 3000 years ago) which are used in aqueous solutions. Natural rubber, synthetic rubbers, and rubber conversion products, dispersed mostly in aliphatic hydrocarbons, but the choice of solvent is on the basis of economy when other considerations may permit. Cellulose derivatives, vinyl resins, polymers of acrylic and methacrylic esters and shellac form part of solvent activated adhesives[12].

1.6.3 HEAT-SEALING ADHESIVES

These are normally inactive solids at normal temperature but may be converted by application of heat to a sticky or molten condition. They possess valuable properties such as toughness, flexibility, good electrical qualities, . resistance to moisture, solvents and chemicals in general. Waxes have been in use for a long time. Cellulose esters and ethers give excellent thermoplastics. Polyvinyl esters and acetals, have widely different properties and range from the fairly soluble types having extremely low melting points, to those which have medium to high melting points, which are insoluble even in chlorinated solvents. Acetylized vinylpolymers, are used in laminating wherever their heat-sealing and elastic properties are of value. In all cases looked in above the polymeric material is used as the tough film base which gives extra adhesive bonding strength, while waxes and plasticizers, usually of the ester type, are often relied upon to give proper flow at a suitable heat-sealing temperature. Tackifying resins are often added to the intimate mixture to modify the consistency, to give additional adhesion to particular surfaces, and to improve the attachment to the carrier sheet (as cellophane) with which the heat sealing adhesive is used[32].

1.6.4 PRESSURE-SENSITIVE ADHESIVES

Pressure sensitive adhesives attained a role of industrial importance in the early 50's. They differ from the other types of adhesives in that they function immediately on application without any physical or chemical change in the adhesive layer being involved. A pressure - sensitive adhesive must have tack, internal strength, resiliency, cushioning power, and usefulness over a wide range of temperature. To impart these properties, a film-forming, elastomeric or rubber-like material is a highly essential ingredient. Before 1941 crude rubber formed the essential ingredient, but synthetic rubber and other elastomeric resins found greater use. Most of these were tapes but solutions and aqueous dispersion are available.

1.6.5 CHEMICALLY REACTIVE ADHESIVES

Chemically reactive adhesives are those characterized by the occurence of chemical change during the formation of the bond. Three types of reaction are ordinarily encountered in practice:

(1) Cross-linking changes, in which a very small amount of added reagent effects major changes in the solubility and softening point of the adhesive. Common examples are tanning of protein adhesives and the vulcanization of rubber[35].

(2) Condensation, in which growth of molecular size occurs, and a by-product of the reaction is liberated. The curing of phenol-formaldehyde, urea-formaldehyde, and similar resins examplifies this type of reaction.

(3) Polymerization, in which molecular growth occurs by a combination of unit structures. Styrene, acrylic esters and methacrylic esters provide examples. Profound

changes in rheology and chemical activity occur in reactive adhesives before the bond becomes operative.

1.6.6 TESTING OF ADHESIVES

In general, adhesive tests can be classified into quality tests and stability tests.

1.6.7 QUALITY TESTS

These measure the condition of the adhesive bond at the time the test is made. These tests are

(i) Tensile tests which measure the force required to separate the bonds

(ii) Shear tests which measure the force required to cause sliding of the adhesive bonded materials.

(iii) Peel-back, impact, and strip-down tests.

1.6.8 STABILITY TESTS

These are concerned with the condition of the bond and of the adhesive as it would appear after prolonged storage or use. Many of the tests involve prolonged exposure of the adhesive or the bond to elevated or low temperatures, to oxygen and to other chemicals which may deteriorate the adhesive or bond[32].

1.7.0 OBJECTIVES

The objectives of this research were as follows

1. To isolate and characterize vernonia oil from the seeds of Vernonia galamensis species,
2. To convert vernonia oil into dicarboxylic acids (Dibasic acids), and
3. To form resins using vernonia oil and an attempt to form adhesives.

CHAPTER 2

RESULTS AND DISCUSSION

2.0.0 EXTRACTION OF VERNONIA OIL

2.0.1 SEED TEMPERING (LIPASE INACTIVATION)

The result of the TLC analysis of oils from seeds differently tempered are shown in Tables 2.1, 2.2 and 2.3. The solvent system used in all the cases was n-hexane: diethyl ether (70:30 v/v).

TABLE 2.1 OVEN HEATED SEEDS [‡]

SPOT NUMBER	R_f VALUE
1	0.81
2	0.72
3	0.59
4	0.49
5	0.39
6	0.32

[‡] The oven temperature was maintained at about 120°C.

TABLE 2.2 STEAM TEMPERED SEEDS

SPOT NUMBER	R_f VALUE
1	0.66
2	0.49
3	0.39
4	0.33
5	0.20

TABLE 2.3 AUTOCLAVE TEMPERED SEEDS

SPOT NUMBER	R_f VALUE
1	0.84
2	0.57
3	0.44
4	0.33

These experiments indicate clearly that the amount and distribution of free fatty acids depended on the method used for tempering the seeds. Control of the enzymatic lipase activity before vernonia seeds are crushed is necessary[3], this minimizes the amount of free fatty acids in the oil extracted. The major spots as reported in the literature[3] gave the following *Rfs*: normal triglycerides (0.6), monovernoyl triglycerides (0.5), divernoyl triglycerides (0.4), trivernolin (0.3), and vernolic acid (0.25). Oven heated and autoclaved seeds show no spots for vernolic acid (free fatty acid).

2.0.2 PURIFICATION PROCESS OF THE OIL

The crude oil, in all cases, was first refined with activated charcoal and then degummed. It was observed that much oil was lost in this stage of purification. In fact a loss of up to 20 % was observed in one case. Also the use of 2% activated charcoal by weight was not sufficient to decolorize the oil completely thus about 5% by weight was used. Degumming of the oil was done by stirring the oil with some small percentage of distilled water at a ratio of 21:1 (v/v) about 60°C for an hour, followed by centrifugation.

2.0.3 IDENTIFICATION OF THE OIL

Infrared studies performed on several oil samples show significant absorptions at 2900 cm^{-1}, 1740 cm^{-1} 1650 cm^{-1} and 825 cm^{-1}. The absorption around 1650 cm^{-1} is due to the -C=C- stretch vibration and the one at 825 cm^{-1} indicates the presence of the epoxy group. From the physico-chemical data determined below, and the chemical reactions performed [Sections 2.0.4 to 2.1.5] the oil contains the epoxy group and unsaturation as expected of the vernonia oil.

Gunstone's classical degradative studies indicated that Vernonia oil contained vernolic acid (12,13-epoxyoleic acid). Morris and Wharry[35] established vernolic acid to be cis-12-D,13-D-epoxy-cis-9-octadecenoic acid. It is remarkable that cis-12,13-epoxyoleic acid occurs naturally in both its optically active forms. A number of species of the *Compositae, Dipsacacea, Euphorbiacae, Onagracea* and *Valarianaceae* families contain the (+) acid (vernolic) which gives rise to (-),-threo-12,13-dihydroxyoleic acid by acetolysis. The (-)-epoxy acid is a constituent of the seed oils of several *Malvaceae*. This acid on cleavage gives (+)-dihydroxyoleic acid enantiomer.

2.0.4 FREE FATTY ACID DETERMINATION

In order to determine the amount of free fatty acid in the oil the acid value was calculated. This is the amount in milligrams of KOH which would neutralize free fatty acids in 1 g of the sample[17]. The acid value is calculated from the following expression:

$$\text{Acid value} = \frac{V \times 56.1 \times A}{W}$$

Where

V = the volume of alkali used

A = the alkali factor

W = the dry weight (g)of the oil

56.1 = weight in grams of KOH.

TABLE 2.4 ACID VALUE DETERMINATION

VOLUME OF KOH (CM^3)	WEIGHT (g)	ALKALI FACTOR	ACID VALUE (mg)
2.3	0.597	0.0100	2.16
2.5	0.626	0.0100	2.24
2.4	0.585	0.0100	2.30
2.6	0.691	0.0100	2.11

2.0.5 UNSAPONIFIABLE MATTER

Unsaponifiable matter may be liquid or solid. Those of the liquid type are normally made up of hydrocarbon oils, tar oils or rosin oils. The solid part includes paraffins cresene (refined ozokonite), higher alcohols, cholesterols or lactones. The unsaponifiable matter averages 1.170% of the oil.

TABLE 2.5 DETERMINATION OF UNSAPONIFIABLE MATTER

WEIGHT OF OIL (g)	WEIGHT OF UNSAPONIFIABLE MATTER (g)	PERCENTAGE OF OIL UNSAPONIFICATION
10.10	0.420	4.1584
9.98	0.417	4.1784
9.99	0.418	4.1792

2.0.6 SAPONIFICATION VALUE

The alkali factor used for this analysis was determined by placing 0.5 M HCl in a burette and titrating against 25 cm^3 of alcoholic KOH accurately pipetted into a conical flask. This value was 0.4694.

TABLE 2.6 SAPONIFICATION VALUES

WEIGHT OF OIL USED	SAPONIFICATION VALUE	SAPONIFICATION EQUIVALENT
2.103	170.776	328.500
2.098	173.808	322.770
2.097	172.007	326.150
2.100	171.980	326.201
2.099	172.342	325.516

The results show that the oil had saponification equivalent similar to that obtained for *V.anthelmintica* seed oil (322.9)[22]. Saponification equivalent which is related to the saponification value by the formula[17]:

$$\text{saponification equivalent} = \frac{56\ 100}{\text{saponification value}}$$

affords a fairly clear indication of the mean molecular magnitude of an ester (triglyceride or otherwise) which is present.

2.0.7 THE IODINE NUMBER

Dry potassium dichromate accurately weighed was dissolved and diluted to 250 cm^3 with distilled water. This was standardised with half-molar potassium thiosulphate. Starch solution was used to mark the end point. The colour changes were from reddish brown to green then blue-black which finally settled as pale blue.

TABLE 2.7 VOLUME OF THIOSULPHATE USED

Weight of oil used (g)	Volume of thiosulphate for the blank (V_b)/cm^3	Volume of thiosulphate for the sample (V_s)/cm^3
0.2505	22.2	20.3
0.2503	22.2	20.3
0.2509	22.2	20.3

TABLE 2.8 IODINE NUMBER CALCULATION

RUN NUMBER	WEIGHT OF OIL (g)	IODINE NUMBER g/100g
1	0.2114	86.4
2	0.2105	86.5
3	0.2103	86.4
4	0.2109	86.4

The iodine value is given as

$$\frac{(V_b - V_w) \times 127.92 \times I}{W}$$

Where 127.92 is the atomic mass of iodine,

I, the actual concentration of sodium thiosulphate,

V_b, the volume of thiosulphate for the blank and,

V_w, the volume of thiosulphate for the sample.

Vernonia oil from *Vernonia anthelmintica* had an iodine number of 107.5[39] and so we see that the iodine number determined for our oil, (86.4) is lower.

2.0.8 ESTIMATION OF EPOXIDES

Epoxides were identified by the picric acid method. The oil sample contained the epoxy ring as was indicated by the four experiments which were performed. The epoxides appeared as orange spots on a yellow background. In the estimation of the percentage of the epoxide present in the oil, the following equation was used.

Percentage oxirane oxygen is given by

$$\frac{V_1 - (V_2 - V_3) \text{ X } N_1 \text{ X } 16 \text{ X } 100}{W \text{ X } 1\ 000}$$

Where

V_1 is volume (ml) of NaOH solution for blank

V_2 is volume (ml) of NaOH solution for the sample

V_3 is volume (ml) of NaOH solution for the titration of the free acid in the sample.

N_1 is the normality of NaOH

W is the weight of the oil sample

16 weight of oxygen.

TABLE 2.9 FREE ACID DETERMINATION

Volume of NaOH For blank (V_1) /CM^3	Volume of NaOH For sample (V_2) /CM^3	Free acid (V_3) /CM^3
20.3	12.4	2.1
21.1	12.9	2.5
20.6	12.4	2.3
22.4	13.1	3.8

TABLE 2.10 PERCENTAGE OXIRANE

RUN NUMBER	PERCENTAGE OXIRANE NUMBER
1	3.817
2	4.060
3	3.941
4	3.998

The percentage oxirane for oil from *Vernonia anthelmintica* was found to be 4.0[39]. The reaction taking place between the epoxy group and the hydrogen halide can be envisaged to be one of ring opening and halogen addition. It has been shown[40] that this hydrohalogenation of oxirane compounds is quite specific.

2.0.9 VISCOSITY OF VERNONIA OIL

Viscosity is mostly expressed either as absolute viscosity or as relative viscosity. Therefore, it was necessary to calculate both the absolute and relative viscosities for the oil. The time flow for the distilled water and vernonia oil was recorded (table 2.11). The density and viscosity values for distilled water were obtained from the Handbook of Chemistry and Physics[36].

TABLE 2.11 MEASURED TIME FLOW FOR THE OIL SAMPLES AND WATER

RUN NUMBER	SAMPLE FLOW TIME (Sec.)	DISTILLED WATER FLOW TIME (Sec.)
1	1701.10	13.26
2	1701.20	13.25
3	1701.00	13.27
4	1701.10	13.25
5	1701.10	13.25
6	1701.20	13.25
7	1701.30	13.25

MEAN = 1701.11s (FOR THE SAMPLE)

MEAN = 13.26s (DISTILLED WATER)

Viscosity for the oil sample was obtained from the expression

$$\eta = \eta_1 \cdot t/t_1 d_0$$

Where η = absolute viscosity of oil

η_1 = absolute viscosity of distilled water

t = time of flow of oil

t_1 = time of flow of distilled water.

d_θ = density of water at temperature θ

Since the density of distilled water at 25°C, d_{25}, is 0.99707 g/ml, and the viscosity of the distilled water at 25°C, η_{25}, is given as 0.8937 centipoises then

$$\eta_{25} = \frac{0.8937 \text{ X } 1701.1 \text{ X } 0.953088}{13.26 \text{ X } 0.99707}$$

$$= 109.974 \text{ cps}$$

Therefore 109.974 cps is the absolute viscosity of vernonia oil at 25°C. Its viscosity relative to that of water is obtained by omitting the absolute viscosity of water in the above calculation. The kinematic viscosity V is given by the absolute viscosity divided by the samples' density,

$$V = \frac{\eta}{D}$$

Substitution gives 120.665 stokes.

2.1.0 HYDROLYSIS OF VERNOLIC ACID

The yellow suspension on hydrolysis gave a mixture of fatty acids with vernolic (cis-12, 13-epoxy-cis-octadec-9-enoic) acid being 80.3% of the total, as indicated by gas chromatography which gave five peaks. By comparison, five fatty acids- 79.81% vernolic, 10.23% linoleic, 5.3% oleic, 2.22% stearic, and 2.37% acids- have been reported from vernonia oil of *Vernonia galamensis* seeds grown in Zimbambwe[37].

2.1.1 CONVERSION OF VERNOLIC ACID TO 12,13-DIHYDROXYOCTADEC-9-ENOIC ACID

Vernolic acid (8 g) after acetylation for 8 hours and subsequent hydrolysis produced fine crystals of 12,13-dihydroxyoctadec-9-enoic acid [6.2 g, 75% yield, RRK-1, after purification and recrystallization, achieved by use of diethyl ether-ethanol mixture (40:60 v/v)]. The mass spectrum had molecular peaks characteristic of a fatty acid. It contained the fragment of mass 60 which is largely due to McLafferty's[38] rearrangement to give $CH_2=C(OH)OH^+$. The large peaks at masses 31, 45 and 59 indicate the presence of oxygen. The peak at mass 45 is due to $(COOH)^+$ and always appears shorter than the peaks at masses 31 and 59. The **EI MS** gave the following significant peaks: m/z 356(M)(1), 337(1), 290(1), 267(6), 238(4), 223(12), 209(5), 166(8), 153(14), 129(11),

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2.1.2 PRODUCTS OF PERMANGANATE OXIDATION OF 12,13-DIHYDROXYOCTADEC-9-ENOIC ACID

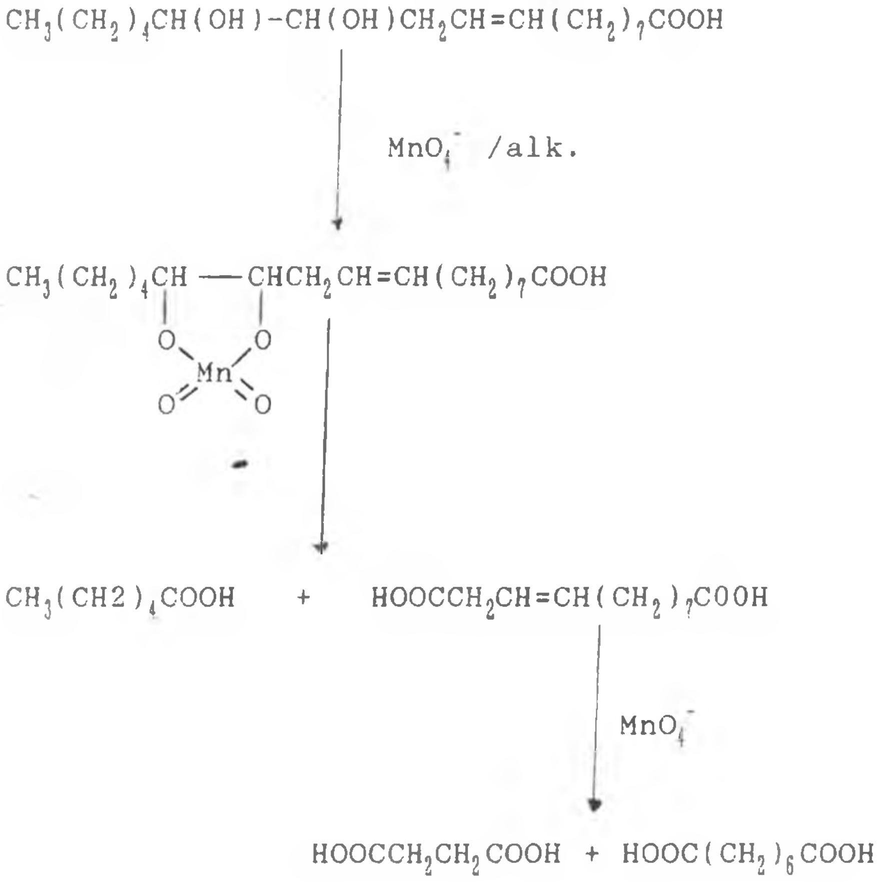
The 12,13-dihydroxyoctadec-9-enoic acid (2g) when oxidised with potassium permanganate gave a strongly smelling liquid, identified as hexanoic acid (b.p. 203°C, literature 202-204°C). Off-white crystals (RRK-2, octanedioic or suberic acid, m.p. 140°C, 360 mg) were obtained. Identification was done by the use of an authentic sample of octanedioic acid. ^1H NMR spectroscopy found δ1.16 (4H, p, J=3.8Hz), δ1.41 (4H, t, J=5.1 Hz), δ2.09 (4H, t, J=7.3 Hz), δ4.78 (2H, broad). The ^{13}C NMR gave the following frequency areas δ25.86 Hz (-CH$_2$-), δ29.84 Hz (-CH$_2$-), δ34.81 Hz (-CH$_2$-) and δ177.90 Hz (Carbonyl carbon). The EI MS gave the following: M/z 157(50), 152(12), 138(90), 128(10), 115(26), 110(29), 97(80), 82(18), 73(55), 69(100), 60(80), 55(95), 45(54), 41(76).

The oxidation resulted in the olefinic cleavage with the shifting of the bond. This type of reaction was found to occur with *V. anthelmintica* oil[43]. The postulated mechanism for the synthesis of octanedioic acid from

12,13-dihydroxyoctadec-9-enoic acid is given in Scheme 2 below.

SCHEME 2

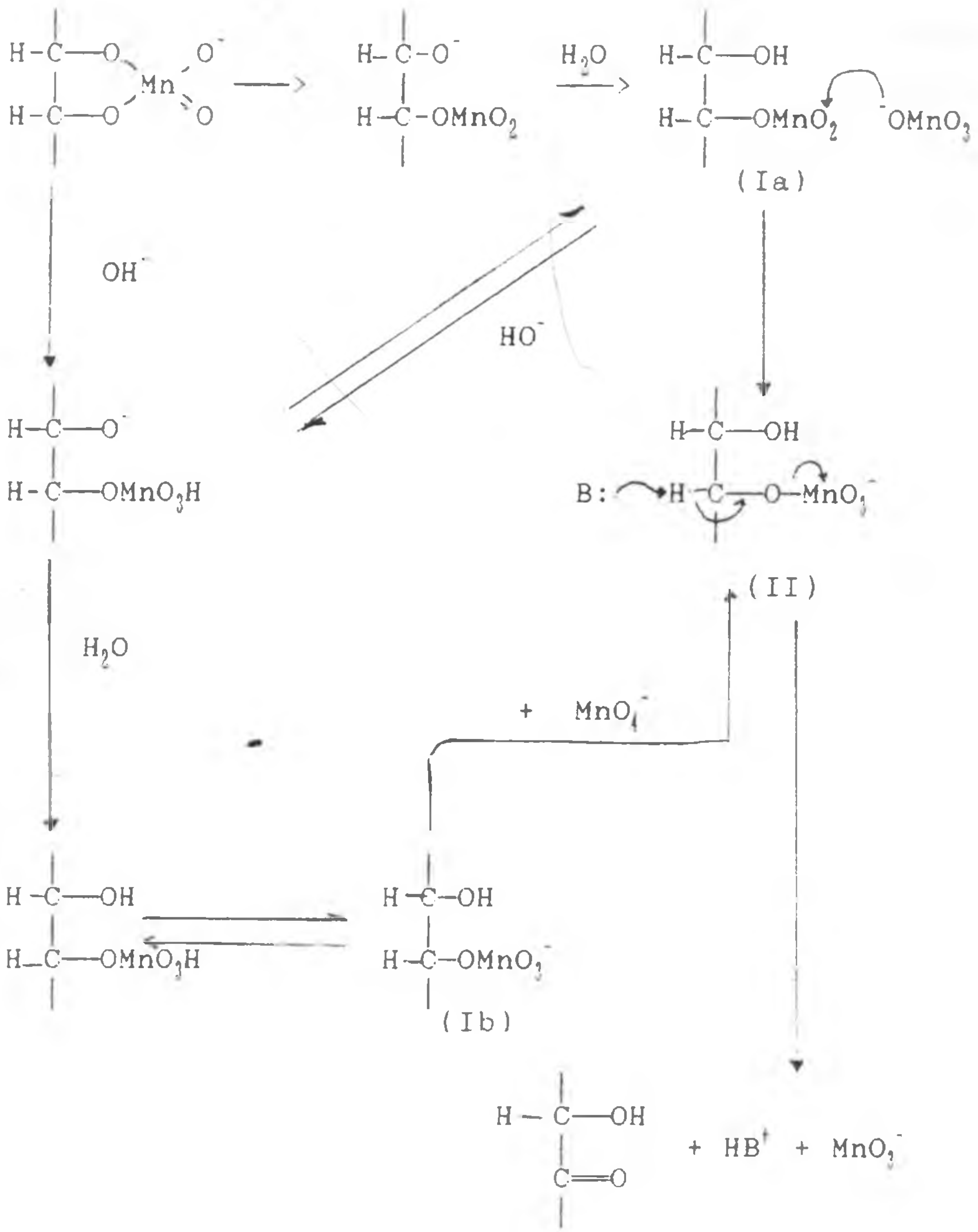
Postulated mechanism for the synthesis of octanedioic acid from 12,13-dihydroxyoctadec-9-enoic acid



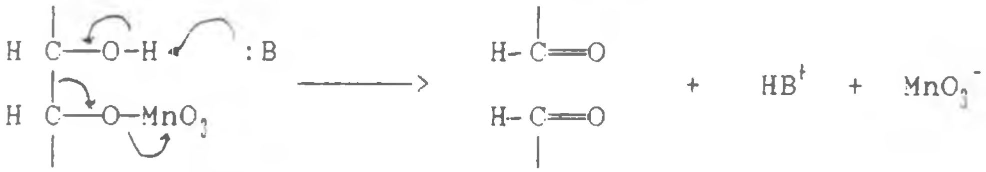
Scheme 3[39] shows the expected route followed in the permanganate oxidation of olefins:

SCHEME 3

The mechanism for permanganate oxidation of olefins



The first step in the hydrolysis of the cyclic permanganate ester would lead to Ia or Ib depending on how the manganese oxygen was broken. The intermediate (Ia or Ib) could be converted to the diol by oxidation of the Mn(V) in the intermediate to Mn (VI) by permanganate, followed by cleavage of the remaining manganese-oxygen bond in such a way as to produce a carbonyl group. Intermediate II (which normally leads to a ketal) could also give the dialdehyde by attack of base on a different position:



Further oxidation of the original cyclic permanganate ester before the initial cleavage might occur. This would lead to an intermediate similar to that in the periodate acid oxidation of diols to carbonyl compounds.

2.1.3 LEAD TETRAACETATE OXIDATION

Vernolic acid (RRK-1) was oxidised with lead tetraacetate in benzene at room temperature. Hexanal was produced as well as fine crystals (RRK-5) which produced a deep orange 2,4-dinitrophenyl hydrazone (mp. 180-181°C) and a semicarbazone. The UV absorption at 238 nm, indicates that there exists a double bond in the compound.

Oxidation with lead tetraacetate has had very wide application in determining the structure of sugars and it has also been applied to the demonstration of the position of hydroxy groups in di-and tetrahydroxystearic acids and in certain cork acids[10]. Criegee[11] has demonstrated that lead tetraacetate in acetic acid at room temperature can cleave vic glycols to aldehydes, ketones or both, according to the structure of the glycol. He also demonstrated that glycol cleavage is characterised by its specificity, speed of reaction and generality of application. For example, 9,10-dihydroxystearic acid was cleaved to yield pelargonic aldehyde and azelaic half aldehyde as shown below:

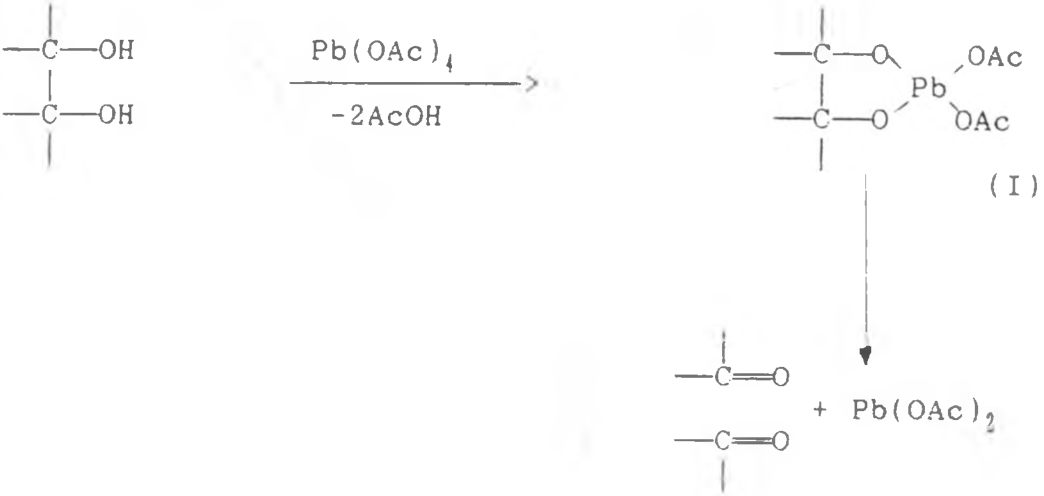
$$CH_3(CH_2)_7CH(OH)\text{-}CH(OH)(CH_2)_7COOH$$

$$\Big\downarrow \; Pb(OCOCH_3)_4$$

$$\underset{\text{Pelargonic aldehyde}}{CH_3(CH_2)_7CHO} \quad + \quad \underset{\text{azelaic half aldehyde}}{OHC(CH_2)_7COOH}$$

The suggested cleavage proceeds through an intermediate cyclic ester (I), as shown in scheme 4 below

SCHEME 4 Suggested cleavage of diols by lead tetraacetate



2.1.4 PRODUCTS OF PERIODATE-PERMANGANATE OXIDATION OF 12,13-DIHYDROXYOCTADEC-9-ENOIC ACID

The oxidation of 12,13-dihydroxyoctadec-9-enoic acid with periodate-permanganate, yielded white flaky crystals, of nonanedioic acid, RRK-3 (178mg, m.p. 103°C). An authentic sample of nonanedioic acid was used for identification. The melting point of this product agrees with literature for nonanedioic acid (mp 102-104 °C). Mass spectrometry gave characteristic peaks of straight chain carboxylic acids. EI MS gave the following significant peaks: 183(0.4), 152(50), 98(24), 83(55), 69(48), 55(100), 41(63). ^{1}H **NMR** (MeOH) spectra gave the following: δ1.15 (s, 6H), δ1.40 (t, 4H, j=6.42Hz), δ2.08 (4H, t, J= 7.42Hz) and δ4.78 (2H, broad). ^{13}C **NMR** (MeOH) gave δ25.97 (-CH$_2$-), δ30.26 (-CH$_2$-), δ34.88 (-CH-), and δ177.89 (C=O). It was possible to show that the fourth carbon had a coincidental peak with the third carbon by using the following formula[42]:

$$D_c(K) = B_s + \Sigma D_m A_{sm} + Y_s N_{k3} + D_s N_{k4}$$

Where B_s, A_{sm}, Y_s and D_s are constants and

N_{kp} is the number of carbon atoms p bonds away from the kth carbon.

D_m is the number of carbon atoms bonded to the kth carbon atom that has m attached carbons.

s is the number of carbon atoms bonded to the kth atom.

Taking the following structure of nonanedioic acid,

$HOOCCH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$

and assuming that the middle $-CH_2-$ act as in an alkane molecule, and that the effect of the carbonyl group on them is not significant, the ^{13}C NMR (MeOH) values for the C-4 and C-5 coincide. Carbon 5: calculated, 30.72 (observed 30.26); carbon 4: calculated, 29.96 (observed 30.26). The parameters used for the calculation of the carbons in the above molecule are for alkanes.

Permanganate-periodate reagent has been reported to have completely cleaved the olefin in oleic acid. In fact it was found that the periodate alone could not oxidize oleic acid. Thus, this reagent depends on the ability of the periodate ions to regenerate permanganate from its reduced state. Lemieux and von Rudloff[43] observed that the addition of sodium meta-periodate to an alkaline solution of manganate ion(II) resulted in the immediate formation of permanganate ion. This reagent is specific for olefinic linkage.

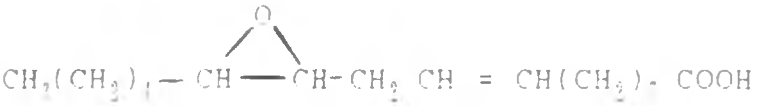
2.1.5 CHARACTERIZATION OF THE 12,13-DIHYDROXYOCTADEC-9-ENOIC ACID

The oxidation of RRK-1 by the use of potassium permanganate, a method known to indicate satisfactorily the position of double bonds, gave nonanedioic acid and hexanoic acid (Section 2.1.2). Another volatile substance was formed which was not identified. Oxidation with permanganate-periodate reagent (Section 2.1.4) yielded nonanedioic acid, an indication that the double bond is in the ninth position. This shows that the product obtained is 12,13-dihydroxyoctadec-9-enoic acid,

$$CH_3(CH_2)_4CH(OH)-CH(OH)CH_2CH=CH(CH_2)_7COOH$$

12,13-dihydroxyoctadec-9-enoic acid

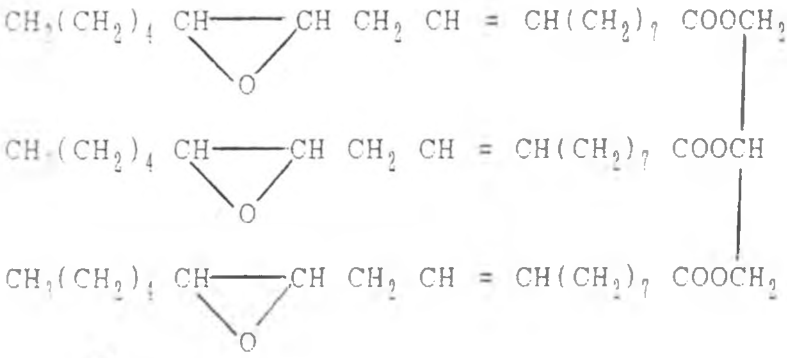
and the original acid must clearly have been 12,13-epoxyoctade-9-enoic acid.



$$CH_3(CH_2)_4-CH\overset{O}{—}CH-CH_2CH=CH(CH_2)_7COOH$$

12,13-epoxyoctadec-9-enoic acid

On oxidation using lead tetraacetate, the acid yielded an unsaturated aldehyde, which gave a deep orange 2,4-dinitrophenylhydrazone. Another oxidation by the use of periodate-permanganate reagent yielded nonanedioic acid. Vernonia oil, from which 12,13-dihydroxyoctadec-9-enoic acid was synthesized, is known to be a naturally occurring epoxidized oil existing as a triglyceride (trivernolin),



2.2.0 RESIN A PROPERTIES

Resin A prepared by refluxing 35% formalin (4.3 g), phenol (10 g), oxalic acid (0.0015 g) and vernonia oil (25 g) was yellow-green in colour. It was soft compared to the original hard translucent material. This shows that the original material was modified. The resin softened when it was heated to about 90°C. It is possible that the oil reacted with the phenol-formaldehyde resin formed to produce a soft elastomer which was not soluble in most of the solvents.

2.2.1 RESIN B PROPERTIES

Resin B prepared by the reaction of vernonia oil with 1,4-Diaminobutane was a soft brown solid which was not soluble in acid or alkali. It was also not soluble in most of the common solvents, except chloroform. Of the many parameters which were to be determined, only three were performed: specific gravity (1.154 g/cm^3), saponification value (30.00 mg KOH/g) and flash point (231.70 °C). It was observed that Resin B had adhesive properties because it stuck on the asbestos tile on which it was spread.

The reaction of a primary amine with the epoxide involves the opening of the epoxide to form an alcohol, without the formation of by-products. This leaves the hydroxyl group available for further reaction and the secondary amine formed then reacts with another epoxy group. Since both the epoxy resin and amine are polyfunctional a highly cross linked structure is created[44].

2.3.0 CONCLUSION AND RECOMMENDATIONS

This study has shown vernonia oil from *Vernonia galamensis* seeds to contain the epoxy group (oxirane number 3.95%), and unsaturation (iodine number 86.4%). The free acid was found to be 2.4 cm^3, viscosity, 110 cps, percentage unsaponification, 4.17 and saponification equivalent, 326.5. Acetylation of the oil with subsequent hydrolysis yielded 12,13-dihydroxy-9-oleic acid, which on oxidation with potassium permanganate reagent, at room temperature, gave hexanoic acid, and octanedioic acid.

Periodate-permanganate oxidation of the 12,13-dihydroxyoctadec-9-enoic acid resulted in hexanoic acid and nonanedioic acid. This shows that olefinic cleavage occurred at the C-9 position. It can be seen therefore that a double bond shift was experienced in the formation of octanedioic acid by the permanganate reagent.

Resin A, prepared by the reaction of phenol, formaldehyde and vernonia oil shows that the oil modified the original phenol formaldehyde resin from a hard, brittle thermosetting plastic to a soft thermoplastic one. Resin B prepared by reacting the oil alone with 1,4-diaminobutane gave adhesive properties. Many properties of these resins were not determined. It would be of interest to find out the other parameters in an attempt to categorically classify these resins. Thus more work should be done on the application of these resins in adhesive formulations.

CHAPTER 3

3.0.0 EXPERIMENTAL SECTION

3.0.1 GENERAL

The melting points were determined using the GALLENKAMP melting point apparatus and are uncorrected. The solvents used throughout were distilled before any extraction, reaction or chromatographic study. Sodium thiosulphate, potassium iodide, iodine, potassium hydroxide and potassium periodate were obtained from Howse and McGeorge, Nairobi, agents of the British Drug House. Iodine trichloride was of analytical grade. Tempered seeds were crushed in a **Wiley Mill Model No. 2**

3.0.2 SPECTROSCOPY

IR study was performed on a PERKIN-ELMER Spectrophotometer, UV recorded on a PYE UNICAM SP8-150 using UV grade solvents and EI MS recorded at 70ev, on a VARIAN 311 machine. ^{1}H NMR recorded at 250 MHz on a BRUKER WM 250 spectrophotometer equipped with 30000 computer at ambient temperature with TMS was used as the internal standard. ^{13}C NMR was recorded at 62.89 MHz on BRUKER WM spectrophotometer. Gas chromatography (GC) of the oil was carried out isothermally at 185°C.

3.1.0 PLANT MATERIAL

Vernonia galamensis was collected from Wangige about 10 kilometres from Nairobi. Other seeds found near Gachie, some 13 kilometres from Nairobi were also used. Some samples for analysis were collected in November 1989 while others were collected in May 1991. Herbarium specimen were deposited at the Herbarium, Botany Department, University of Nairobi.

3.2.0 EXTRACTION AND PURIFICATION OF VERNONIA OIL

3.2.1 SEED TEMPERING (LIPASE INACTIVATION)

Several attempts to inactivate the enzyme normally present in triglyceride oils, were performed. The seeds were either oven heated, steam allowed to pass through them, or autoclaved.

3.2.2 OVEN HEATED SEEDS

Seeds (97g) were oven heated for about 90 minutes. Seed temperature was maintained at 90°C and seed moisture adjusted to about 15%. Tempered seeds were air dried overnight.

3.2.3 STEAM INACTIVATED SEEDS

Dry seeds (187g) were heated at a constant temperature of 100°C by passing steam through them for 2 hours. A sweet smelling pink extract suspected to be essential oils was obtained. The seeds were then left to dry overnight before milling. Oil yield was 37.8%.

3.2.4 AUTOCLAVE HEATED SEEDS

Some seeds (89 g) were put in an autoclave machine and the temperature controlled at 90°C for about 2 hours. The moisture content was kept at about 15%. Degumming and purification process was conducted as above. The oil yield was about 39.7%.

3.2.5 OIL EXTRACTION AND REFINING

The seeds were Soxhlet extracted by the use of pre-distilled n-hexane for about 5 hours. The extract, a dark green oil, was then subjected to rotavaporation in vacuo to remove the solvent. The crude oil was refined by the use of about 5% by weight of activated charcoal, kept at a temperature of 60°C for 1 hour. This was followed by hot filtration using the aid of Celite (80-120 mesh) in a Büchner funnel. The oil, clear brown in colour was degummed by stirring with distilled water in the ratio of 21:1 (v/v) at 50°C for 1 hour followed by centrifugation for 2-3 hours. Gum and oil were separated and the oil dried at 60°C under pressure on a rotary evaporator. The mass of the clear brown oil obtained was 28.205g. This translates to a percentage yield of 21%.

3.2.6 T.L.C ANALYSIS

The oil sample was first dissolved in n-hexane before being spotted on the commercially precoated silica plates. Development was done and the plates were first sprayed with a sulphuric acid chromic acid mixture. The solvent system used was n-hexane: diethyl ether (70:30 v/v). The developed plates were then placed in an oven (at 120°C) and then heated for one to two minutes. The fatty acids appeared as black spots. Another identification skill was employed which involved putting the developed plates in fumes of iodine. The fatty acids appeared as orange/brown spots.

3.2.7 LARGE SCALE EXTRACTION

For large scale extraction, 980 g of milled seeds were used and the method of steam lipase inactivation followed. A clear brown oil (298 g) was obtained after degumming and purification. This translates to an oil recovery of 30.4%.

3.3.0 PHYSICO-CHEMICAL PROPERTIES DETERMINATION

3.3.0.1 FREE FATTY ACID DETERMINATION

The oil (5g) was weighed into a 250 cm^3 flask to which was added 25 cm^3 of methyl alcohol and 25cm^3 of toluene. The solution was freely boiled for two minutes and a few drops of phenolphthalein solution added. Titration was done with vigorous shaking against a solution of approximately decinormal alcoholic potash (the exact strength of which was determined against standard acid) until a semi-permanent pink colour appeared.

3.3.0.2 SAPONIFICATION VALUE

The oil sample (2 g) was weighed accurately into a 250 cm^3 flask and mixed with 30 cm^3 of neutral alcohol. Alcoholic potash (50 cm^3 of semi-molar) was run in from a burette with constant stirring and at a constant rate. The contents of the flask were gently boiled with occasional swirling for one hour under reflux. A blank was also determined at the same time. About 1 cm^3 of phenolphthalein solution was added and the saponified solution and the blank were titrated against standard acid, the difference in the titration volumes giving the amount of alkali consumed in terms of standard acid. Saponification values and saponification equivalent were calculated using the equations below[17].

$$S.V. = \frac{(V_b - V_w)}{W \times 56.1}$$

Where V_b = acid volume required to neutralize KOH in the blank

V_w = acid volume required to neutralize KOH in the saponification mixture,

W = dry weight of the oil.

3.3.0.3 UNSAPONIFIABLE MATTER

The oil (10 g) was saponified by alcoholic potash as in the saponification equivalent test. The alcohol was distilled off and the residue taken up in about 200 cm^3 of water and placed in a 500 cm^3 separating funnel. Ether (200 cm^3) was added, and the whole shaken gently with a horizontal swirling motion. Undue emulsion formed and a few drops of alcohol and brine were added to reduce this effect. When the ethereal and aqueous layers had separated sharply, separation was effected and the aqueous layer re-extracted twice in the same manner with the same volume of fresh ether. The combined ether extracts were washed once with water to remove traces of soap, and then concentrated and finally heated in an oven (at about 120 °C) in a tared flask until the weight was constant.

3.3.0.4 ACETYL VALUE

The acetyl value is the number of milligrams of KOH required to neutralize the acetic acid produced by hydrolysis of one gram of an acetylated oil or fat. It is thus an indication of alcoholic hydroxylic groups (although care must be taken, in cases where free fatty acids are present, that carboxylic hydroxyl is not included in the estimation owing to the production-and incomplete hydrolysis with water- of mixed acetic fatty acid anhydride during acetylation). The sample (10 g) was boiled with acetic anhydride (20 g) for two hours under reflux. The cooled product was poured onto 500 cm^3 of hot water in a beaker and boiled gently for half an hour in presence of a little porous pot. The aqueous layer was then drawn off and the washing process repeated similarly three times, until the aqueous layer was fairly neutral to litmus paper. The acetylated oily layer was then filtered through dry paper and dried.

The dried acetylated product (about 5 g) was then saponified exactly as in the determination of the saponification value of the oil; but when hydrolysis was complete the alcohol was distilled off and the soap taken up in water. The acetic acid, which is now present as potassium acetate was isolated by the distillation method. This was where the aqueous soap solution was rendered free of acid with dilute sulphuric acid and steam distilled until 100 cm^3 of distillate required no more than 0.1 cm^3 of decinormal KOH for neutralization. A total of 600 cm^3 of condensate was required.

3.3.0.5 PREPARATION OF STARCH SOLUTION

Starch was required for the determination of iodine value. Starch as ordinarily supplied has granulose enclosed in an envelope of starch cellulose. It is thus important to use boiling water in an attempt to set free the granulose. The solution of starch was made by filtering a solution of starch to remove any cellulose fragments, then precipitating the starch solution by alcohol. The white powder obtained was collected and dried. It was then boiled with water to yield a liquid which gave a blue colour with a drop of iodine solution.

3.3.0.6 IODINE VALUE

The iodine value is a measure of the degree of unsaturation in a compound. The result is commonly expressed as percentage iodine absorbed. This means the number of grams of iodine per 100 grams of the sample, whether or not the halogen used is actually iodine. Wijs' and Hanus' methods are the common procedures used in the determination of the iodine number, with differences being very small for fats of low iodine value, but is often 2 % or more for those having an iodine value of more than 100. Hanus' method gives a considerably higher value for conjugated oils. Wijs' values obtained for non-conjugated oil are believed to correspond to actual unsaturation. The Wijs' solution was used in the determination of the iodine number in this experiment. The following were the solutions required for the determination.

(i) Decinormal sodium thiosulphate

(ii) 10% potassium iodide solution

(iii) Pure dry carbon tetrachloride or chloroform

(iv) Wijs' solution.

3.3.0.7 PREPARATION OF POTASSIUM DICHROMATE SOLUTION

Pure potassium dichromate (10 g) was ground to a fine powder and then dissolved in a small quantity of water. The solution was filtered through a hot funnel to prevent any early crystallization as the solution was filtered. Finely granulated crystals were formed by cooling the solution. The crystals obtained were left to dry for 2 days after which they were heated in a porcelain dish care being taken to avoid melting. The dry dichromate was stored in a stoppered sample tube. The salt (4.903 g) was accurately weighed and dissolved in distilled water to make a one litre solution.The solution was then kept in a stoppered bottle.

3.3.0.8 DETERMINATION OF IODINE NUMBER

The accurately weighed oil (0.25 g) was placed in a wide necked bottle and dissolved in 15 cm^3 of pure chloroform. Wijs' solution (25 cm^3) was added and the bottle well shaken after inserting the stopper moistened with a drop of potassium iodide solution. This was set aside in the dark for the addition process to proceed to completion (60 min). The solution was then mixed with 15

cm^3 of 10% KI solution and titrated with the standard thiosulphate solution using starch as the final indicator. This was better achieved with constant vigorous shaking to ensure extraction of all the iodine from the organic layer until the liquid became yellow. The starch solution was then added. Blank determinations were done.

3.3.0.9 IDENTIFICATION OF EPOXIDES

Developing system used was petroleum ether (40-60°C)-diethyl ether-acetic acid (72:25:1 v/v/v). After the development, the plates were sprayed thoroughly with 0.005 M picric acid in 95% ethyl alcohol and immediately placed in a tank saturated with the vapour from diethyl ether-95% ethyl alcohol-acetic acid solution (80:20:1 v/v/v). Thirty minutes later, the plate was removed and exposed to fumes of ammonia for two minutes. Epoxides appeared as orange spots on a yellow background[45].

3.3.1.0 ESTIMATION OF EPOXIDES

The hydrochloric acid-dioxan reagent (25.0 cm^3), prepared by mixing 25 cm^3 of concentrated HCl with 100 cm^3 of pure dioxan, [which had been purified by leaving it in contact with the solid KOH for 5 days, followed by fractional distillation] was pippeted into a 50 cm_3 glass stoppered flask. The oil sample (0.2 g) was weighed into the flask. The reaction mixture was allowed to stand at room temperature for 15 minutes and 25 cm^3 of neutral ethyl alcohol added. The excess acid was titrated against the standard 0.1 M methanolic sodium hydroxide. The colour of the indicator changed from pink to yellow just before the end point and from yellow to violet at the end point.

A blank titration was performed with 25.0 cm^3 of the hydrochloric acid-dioxan reagent: the difference between the titration of the blank and of the sample is the volume of sodium hydroxide solution equivalent to the acid consumed by the epoxide. The sample contains free acid, and so the free acid content was determined by dissolving a 0.2 g sample of the compound in 40-50 cm^3. of neutral ethanol and titrating with the standard 0.1 M methanolic sodium hydroxide. The result was calculated as the volume (cm^3) of the standard alkali for the weight of sample employed in the epoxy determination. The percentage of oxirane oxygen was calculated from the formula[23]:

$$\% \text{ Oxirane oxygen} = \frac{V_1-(V_2-V_3) \times N_1 \times 16.00 \times 100}{W \times 1000}$$

Where V_1 = volume (cm^3) of sodium hydroxide solution
used for the blank

V_2 = volume (cm^3) of sodium hydroxide solution used for the sample

V_1 = volume of sodium hydroxide solution used for the titration of the free acid in the sample

N_1 = normality of the sodium hydroxide solution and

W = weight of the sample.

3.3.1.1 DENSITY DETERMINATION

To eliminate any uncalled for errors the pycnometer was thoroughly washed with deionized water and dried before and after weighing. The oil to be analysed and deionized water were both placed in a thermostat to equilibrate their temperatures. The clean pycnometer was then filled with water, with any excess being removed by touching the end with a filter paper. The weight of water was then determined by the use of a Mettler H 10 balance. This process was used to determine the weight of the oil. The pycnometer volume was calculated from the equation[44]:

$$V_p^{25} = \frac{W_w^{25}}{d_w^{25}}$$

Where	W_w^{25}	is the weight of water at 25°C
	d_w^{25}	is the density of water at 25°C
	V_p^{25}	is the volume of pycnometer

Density of the oil was calculated from the equation

$$d = \frac{w}{v}$$

Where w is the weight of the sample and v the density of the pycnometer.

3.3.1.2 DETERMINATION OF VISCOSITY

To ensure that the driving head of the liquid in the Ostwald's viscometer was constant the instrument was aligned in a vertical position throughout. The oil sample contained in a flask was left in a thermostat water-bath to ensure that no error was made due to volume changes. This also reduced the time required for equilibration. A temperature equilibrium was determined by introducing a fixed volume of oil into the viscometer and leaving for 30 minutes. Any air bubble was eliminated by use of a suction pump. The oil was raised approximately one centimetre above the top mark by use of air pressure on the side arm. This pressure was then released and as the oil meniscus slid past the upper mark, the stop watch was started and the time for transit between the top and lower mark taken.

3.4.0 SYNTHESES

3.4.1 SYNTHESIS OF VERNOLIC ACID

Vernonia oil (11.44 g) was mixed with 44.27 g of NaOH to which was added 66 cm^3 of water in a round bottomed flask. A magnetic stirrer was put in the flask and the set-up placed on a hot plate. The mixture was heated to 90°C and maintained at this temperature with continuous stirring for 1 hour. A cream cake was observed to form and floated on the mixture. This was left to cool overnight. Ice cold water (50 cm^3) was added to the reaction mixture and acidified to yield a yellow suspension which was filtered to yield an orange-yellow cake[46].

3.4.2 CONVERSION OF VERNOLIC ACID INTO 12,13-DIHYDROXYOCTADEC-9-ENOIC ACID

Vernolic acid (8g), was boiled with 80 cm^3 of acetic acid for 8 hours. A light brown solution was formed. After removal of excess solvent the residue was hydrolysed by boiling molar alcoholic potassium hydroxide for 1.5 hours. The product, which was a cream solution was left overnight. This was found to have solidified and addition of 40 cm^3 of ether resulted in the formation of needle like crystals after acidification. They did not dissolve in the following solvents: diethyl ether, benzene, hexane, petroleum ether, or chloroform. However, a mixture of solvents were attempted and diethyl ether-ethanol (30:70 v/v) gave white monoclinic crystals 6.8 g which apparently were unstable at room temperature. Repeated recrystallization yielded tiny crystals (RRK-1) 6.2 g found to be an acetylated 12,13-dihydroxyoctadec-9-enoic acid. EI MS gave the following significant ions (M/Z): 356(M)(1), 337(1), 290(1), 267(6), 238(4), 223(12), 209(5), 166(8), 153(11), 129(11), 112(9), 84(33), 69(47), 60(50), 57(67), 55(100). IR max (KBr) cm^{-1} 3500 (OH), 1715 (C=O str), 855 (epoxy ring).

3.4.3 OXIDATION OF 12,13-DIHYDROXYOCTADEC-9-ENOIC ACID

To the concentrate of the 12,13-dihydroxyoctadec-9-enoic acid, RRK-1, (2g) dissolved in acetic acid was added potassium permanganate (8g) portionwise and the temperature maintained below 50°C. When all the reagent had been added and the temperature began to fall the mixture was kept at 10°C to 50°C for 3 hours. The solvent was removed under pressure and the mixture suspended in dilute sulphuric acid and decolorized with sulphur dioxide. Steam distillation was performed to separate the mono-and di-acids which were extracted from the volatile and the non-volatile portions. Two distillates were obtained (60-80°C) and (90-95°C). There was left a dark brown crude residue which was treated with boiling water resulting in the formation of a brown black suspension. Filtration yielded a brown solution and a dark brown residue. Recrystallization of the residue gave rise to off white flaky crystals (RRK-2, mp. 140°C.) The **EI MS** had the following significant peaks (M/z): 157(50), 152(12), 139(90), 128(10), 115(26), 110(29), 97(80), 82(18), 73(55), 69(100), 60(80), 55(95), 45(54), 41(76). The higher boiling point liquid was dried over $MgSO_4$ and Na_2SO_4 and then fractionally distilled to yield strong smelling hexanoic acid (RRK-4), boiling at 203°C.

3.4.4 OXIDATION USING LEAD TETRAACETATE

To 1.97 g of the vernolic acid (RRK-1) was added 3 g of lead tetra-acetate and 80 cm^3 of benzene at room temperature. The dihydroxyoleic acid did not fully dissolve. There was seen a brown coloration and more white crystals formed. Vacuum filtration yielded a brown filtrate and white crystals, believed to be lead acetate and were left to dry in an evaporating dish. The filtrate was extracted with 3 x 100 cm^3 portion of water nearly saturated with salt. This extraction was believed to remove the by-product acetic acid while the salt decreased the solubility of the alkoxy-aldehyde in the water. The benzene was removed under pressure. TLC revealed three spots and this product was distilled under pressure to yield three distillates. The third distillate was a compound with a strong smell (RRK-β). Attempted recrystallization gave white crystals which gave UV peak at about 238 nm. The crystals were then reacted with 2,4-dinitrophenyl hydrazine to yield deep orange crystals (RRK-P mp 180-181°C). RRK-P formed white crystals with semicarbazide.

3.4.5 OXIDATION USING PERIODATE-PERMANGANATE MIXTURE

RRK-1 (331 mg) was oxidised in an aqueous solution (150 2cm) containing *tert*-butyl alcohol (90 cm^{3}), the periodate-permanganate reagent (60 cm^{3}) and enough potassium carbonate to give a pH of 8-9. The reaction was stopped after 23 hours by addition of HCl and enough sodium metabisulphate to convert all periodate, iodate and iodine into iodide. The decolorised solution was basified, the butyl alcohol distilled off under reduced pressure, and the remaining solution acidified and continuously extracted with ether. White crystals were obtained after recrystallization from water (RRK-3, 178 mg, mp. 102-103°C). **EI MS** gave the following significant peaks: 183(0.1), 152(50), 98(21), 83(55), 69(18), 55(100), 41(63).

3.5.0 RESIN FORMATION

3.5.1 RESIN A

In a 250 cm flask equipped with a reflux condenser, a thermometer and a stirrer, phenol(10 g) ,37% formalin (4.3 g), oxalic acid (0.0015 g)[47] as a catalyst were mixed and heated in an oil bath. When the temperature of the mixture became 85°C, the heating was stopped and the temperature raised by the heat of reaction evolved to bring about reflux with boiling. After violent reflux had subsided, the mixture was heated again to continue the reaction for one more hour. After the reaction, the product was removed to an evaporating dish and the water layer removed by decantation. There was left a brittle translucent plastic material, which did not change on heating.

In another 250 cm flask equipped with a reflux condenser, a thermometer and a stirrer, phenol (10g), 37% formalin (4.3 g), oxalic acid (0.0015 g) as a catalyst and vernonia oil (25 g), were mixed and heated in an oil bath. The reaction was continued as above and at the end it was observed that the material formed was a soft green plastic material which softened when heated. This was kept as Resin A. It was markedly different from the plastic formed without heating together with the oil.

3.5.2 RESIN B

Vernonia oil, 29.094g (0.033 moles) was mixed with 10.18 g of 1,4-diaminobutane and heated under reflux for about 6 hours but no apparent change was observed. An attempt was made with the use of a few drops of hydrogen peroxide acting as a catalyst. There was an immediate change of colour after the addition of the peroxide. This mixture was further heated for about 12 hours under reflux and on cooling, the mixture formed a brown sticky solid. This was mixed with Na_2SO_4 in an attempt to dry the solid. Distillation was performed to result in a yellow distillate. This was believed to be a mixture of water, diamine, and any volatile compounds, as indicated by the TLC. A brown solid, Resin B, soluble in glacial acetic and chloroform remained.

Resin B was not soluble in water but it dissolved in chloroform. It was then spread on an asbestos tile and after leaving overnight, it was observed that it stuck onto the tile showing that it had some adhesive properties.

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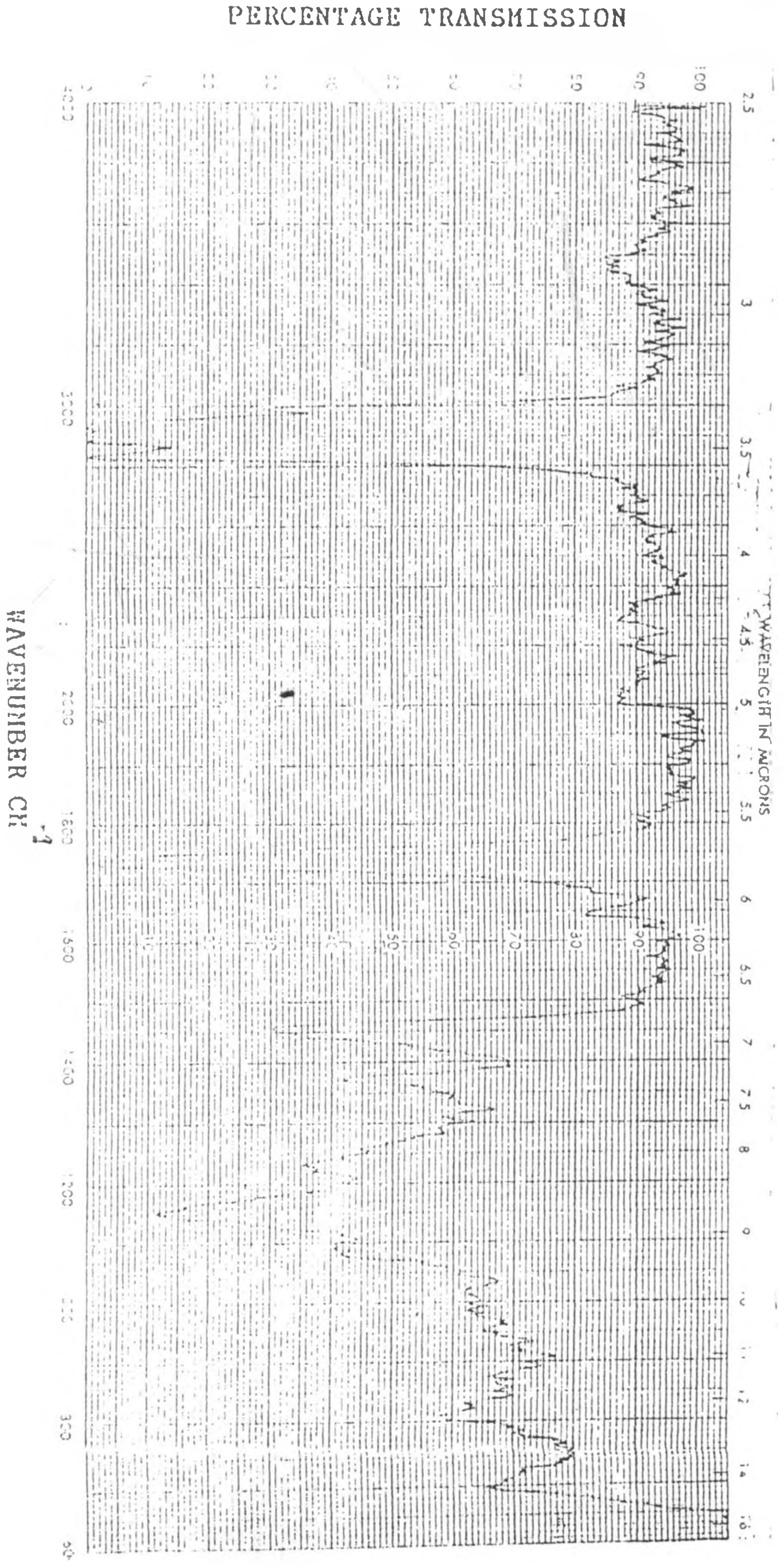
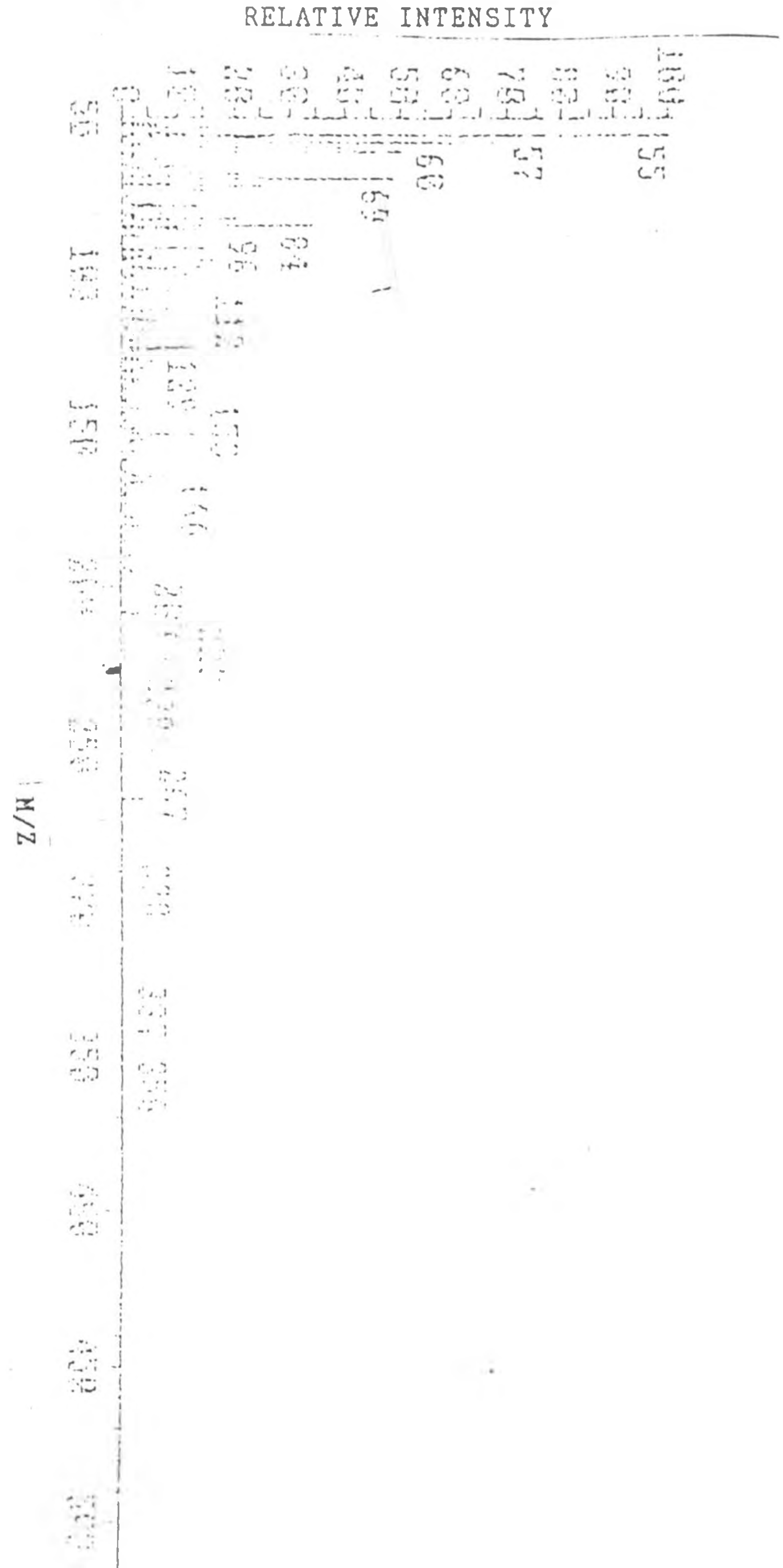
IR SPECTRUM OF VERNONIA OIL



EI MS OF ACETYLATED 12,13-DIHYDROXYOCTADEC-9-ENOIC ACID (RRK-1)



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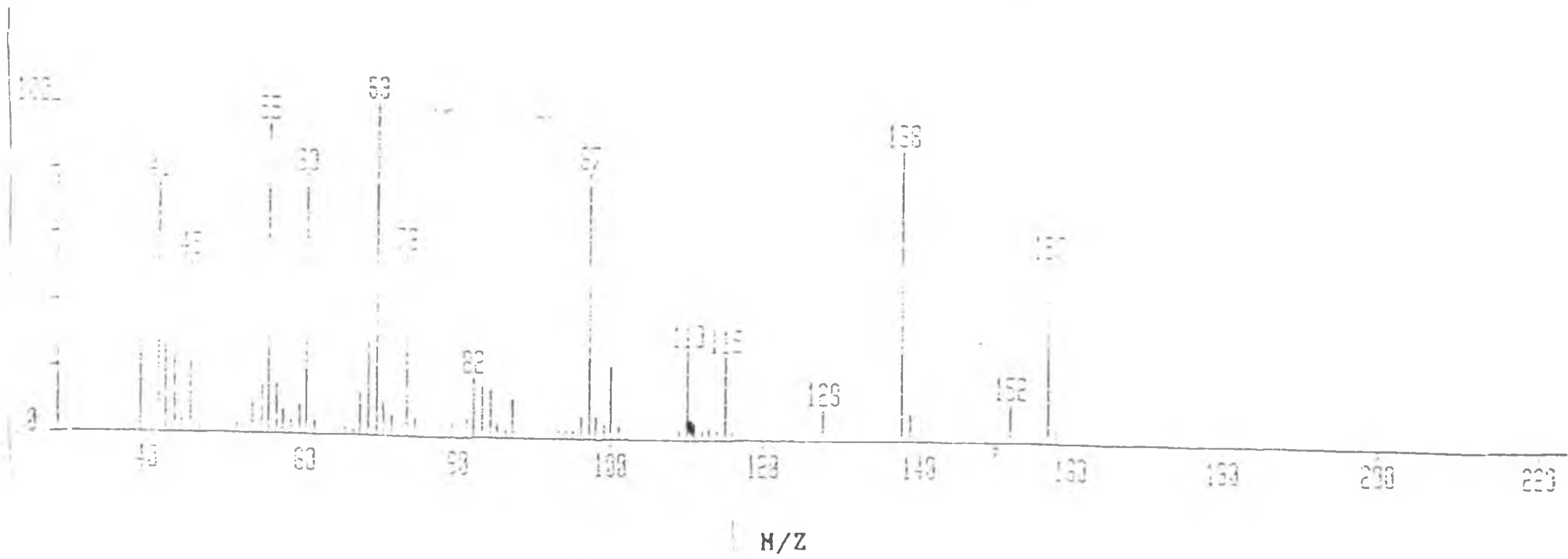

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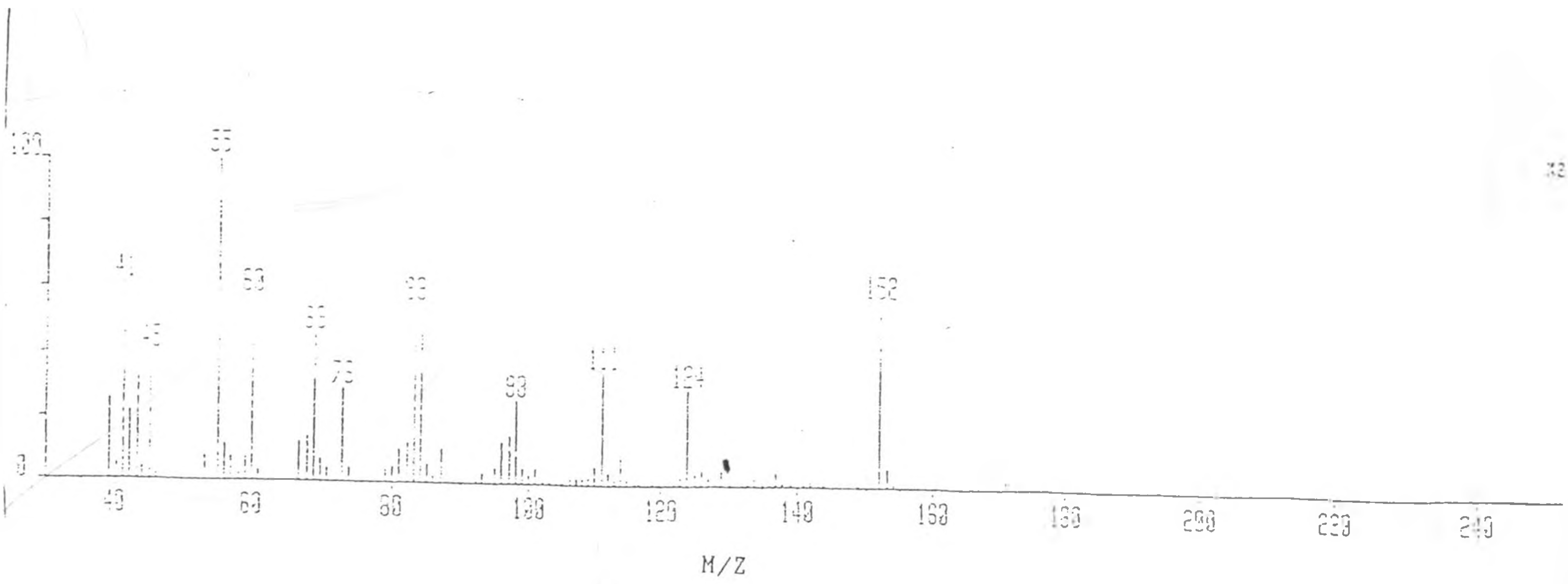

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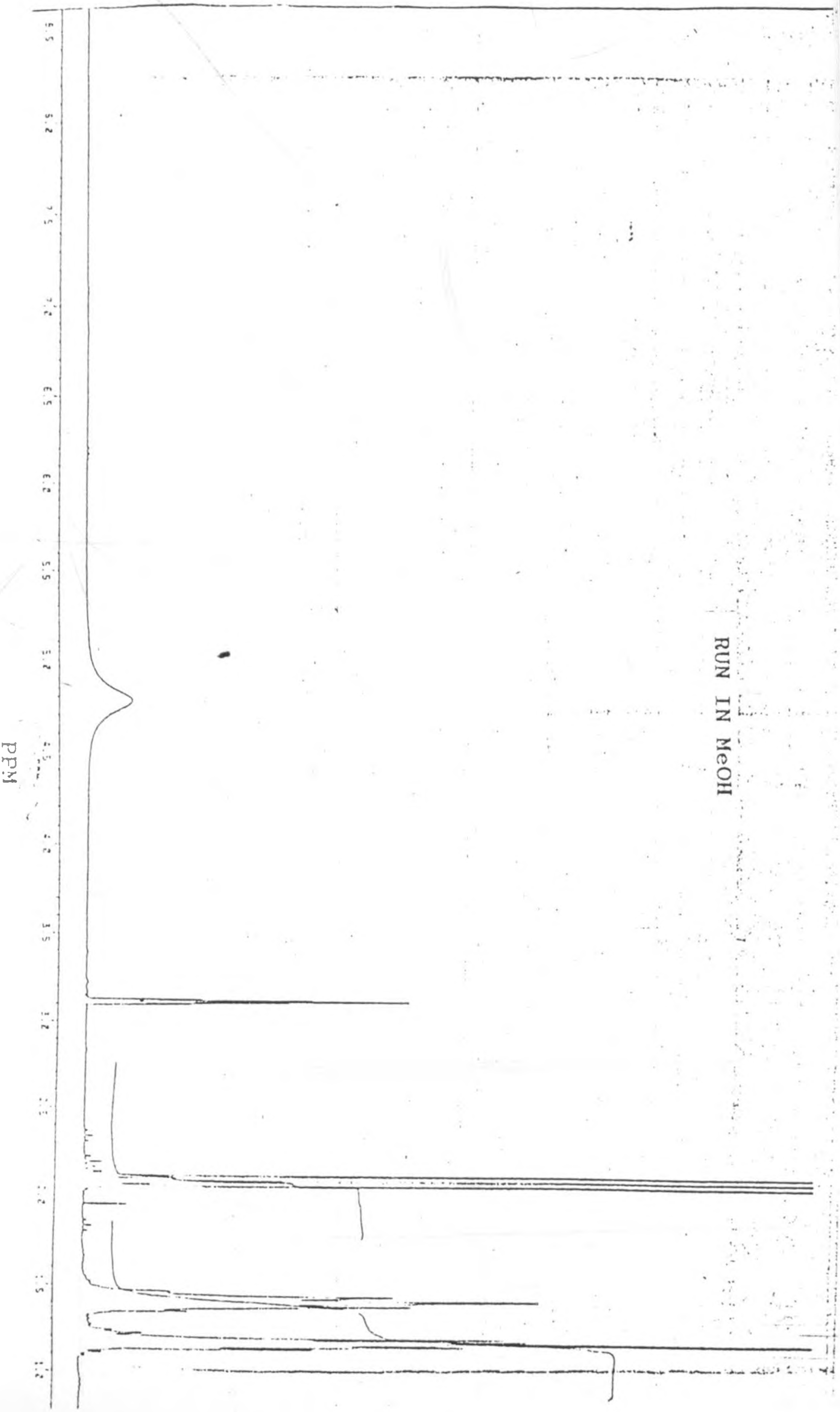

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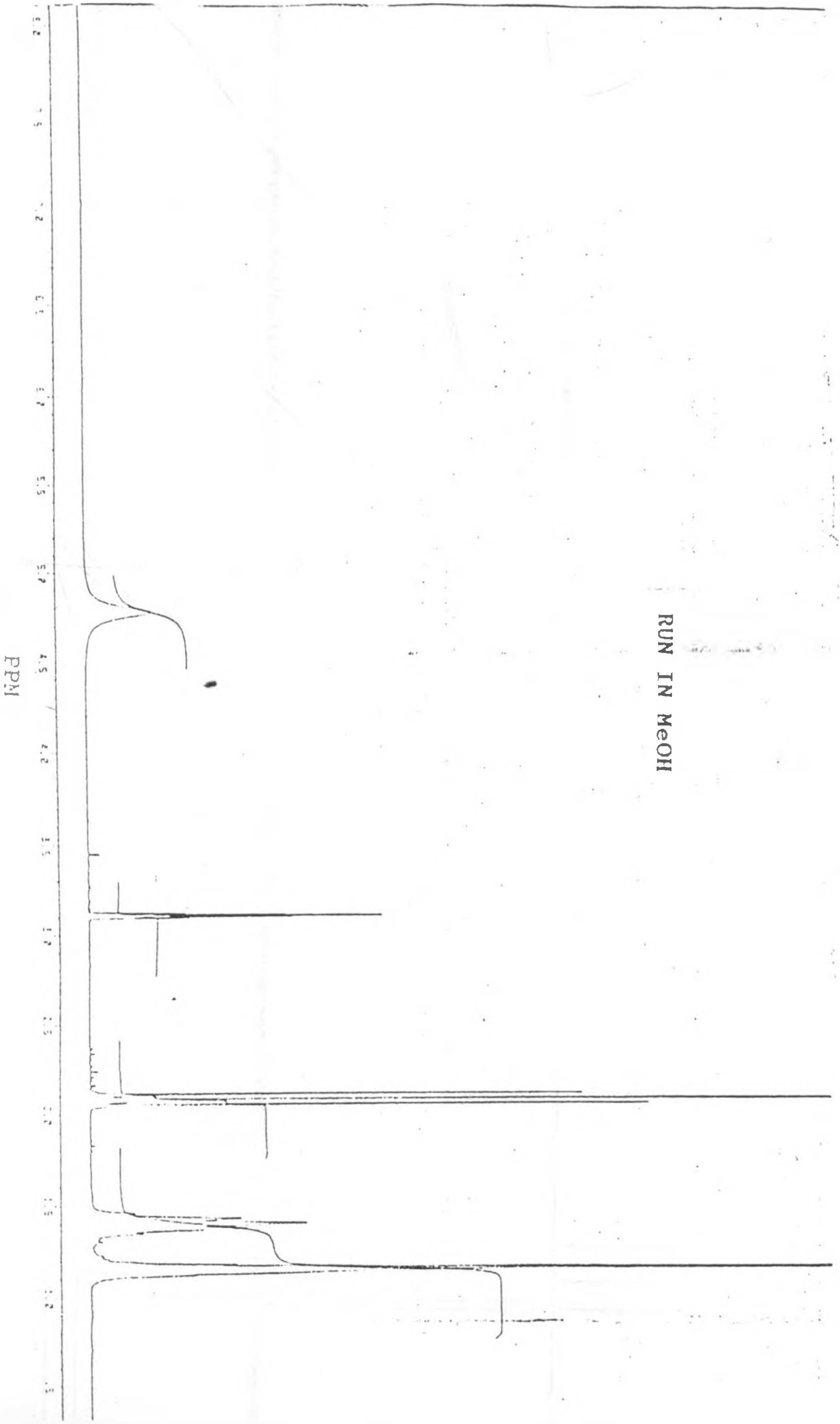
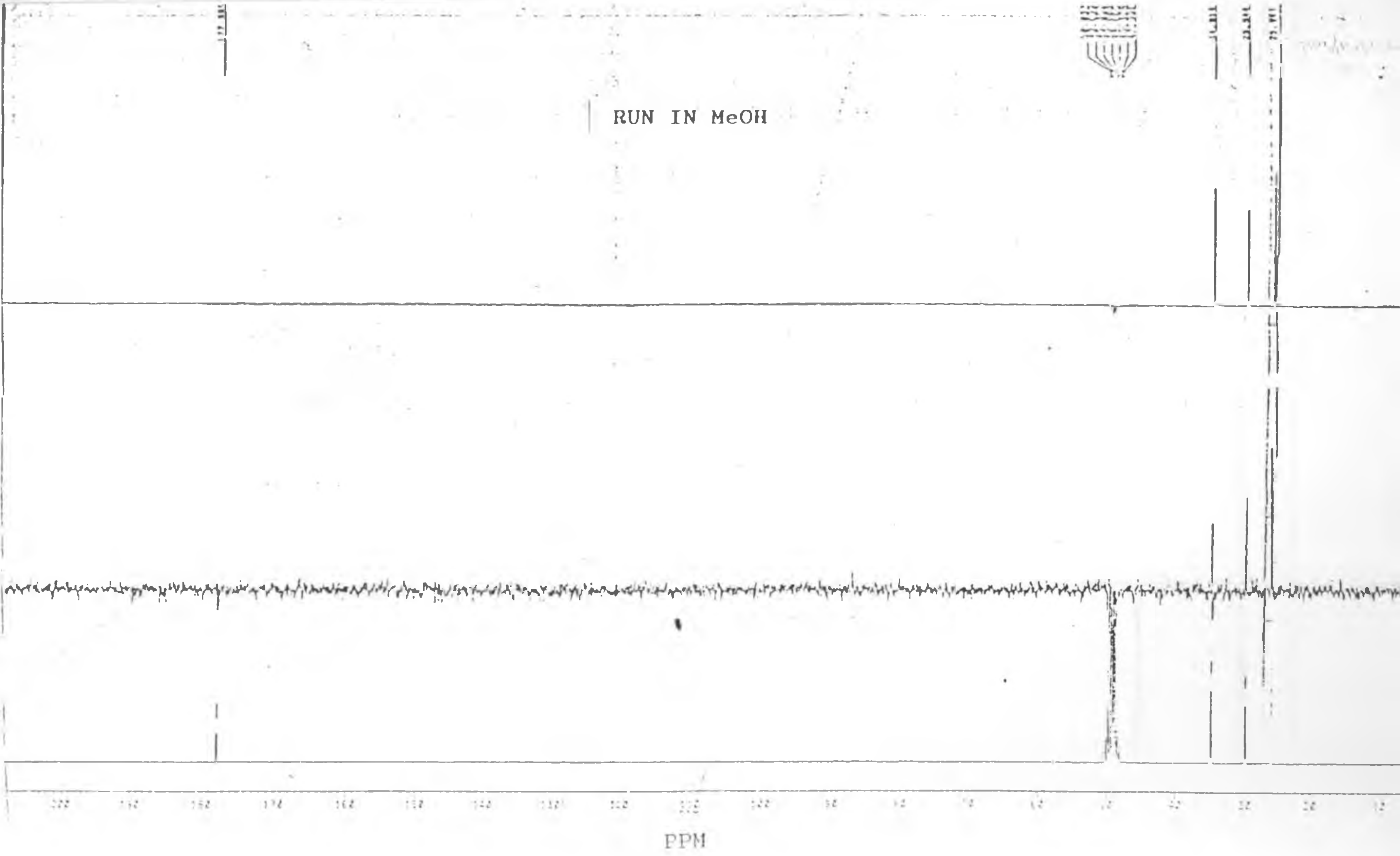


^{13}C NMR SPECTRUM OF OCTANEDIOIC ACID (RRK-2)



^{13}C NMR SPECTRUM OF NONANEDIOIC ACID (RRK-3)