

EXTRACTION AND CHARACTERIZATION OF VERNONIA OIL AND
SYNTHESIS OF N-(AMINOALKYL)VERNOLAMIDES AND N,N'-
POLYMETHYLENE BIS(VERNOLAMIDES).''

BY

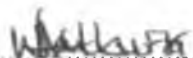
MARY WAWIRA ARTHUR

A Thesis submitted in partial fulfillment for the
Degree of Master of Science of the
[University of Nairobi.]

1996

DECLARATION

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



MARY WAWIRA ARTHUR

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

Supervisors:-

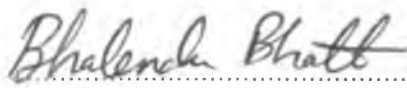


PROF. PETER M GITU

DEPARTMENT OF CHEMISTRY

UNIVERSITY OF NAIROBI

NAIROBI, KENYA



DR BHALENDU. M. BHATT

DEPARTMENT OF CHEMISTRY

UNIVERSITY OF NAIROBI

NAIROBI, KENYA

.....
DR SARINA GRINBERG

THE INSTITUTES FOR APPLIED RESEARCH

BEN-GURION UNIVERSITY OF THE NEGEV

BEER-SHEVA, ISRAEL

DEDICATION

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ABSTRACT

Vernonia galamensis is an annual herb found growing as a common weed in most parts of Africa, but its center of diversity is in East Africa. The dry seed of this plant consists of about 40% naturally epoxidized oil that contains 72-80% vernolic acid (*cis*-12,13-epoxyoctadec-9-enoic acid). This makes this plant an excellent source of naturally epoxidized triglyceride oil. The present study reports on the extraction of vernonia oil and the preparation of epoxy-containing aminoamides and diamides.

The oil was extracted from different varieties of vernonia plant (var. *Nairobensis*; var. *Ethiopica*, var. *galamensis*) using both soxhlet and cold extraction methods. Soxhlet extraction was found to yield more oil (36-40%) that contains low content of vernolic acid, while cold extraction gave a slightly lower yield of oil (20-39%) that contains a high content of vernolic acid. Var *Ethiopica* gave the highest yield in both extraction methods (39-40%), while var. *galamensis* gave the lowest yield (20-26%). Vernonia oil was then reacted with 1,2-diaminoethane; 1,3-diaminopropane; 1,4-diaminobutane and 1,6-diaminohexane to give the corresponding aminoamides [N-(2-aminoethyl)vernolamide, N-(3-aminopropyl)vernolamide, N-(4-aminobutyl)vernolamide, N-(6-aminoethyl)vernolamide] and diamides [N,N'-Ethylene bis(vernolamide), N,N'-trimethylene bis(vernolamide), N,N'-tetramethylene bis(vernolamide) and N,N'-hexamethylene bis(vernolamide)] of vernolic acid.

The reaction of vernonia oil with 1,4-diaminobutane was used to study the following different parameters which influenced aminolysis of the triglyceride:

- (i) Molar ratios of vernonia oil to diamine (1:1; 1:2; 1:3; 1:5; 1:7 and 1:10)
- (ii) Temperatures (room temperature, 50°C, 60°C and 70°C).
- (iii) Reaction period.
- (iv) Solvents (without solvent, chloroform, tetrahydrofuran, carbon tetrachloride, acetonitrile and ethanol).

Highest yields of aminoamide (70-80%) were obtained at room temperature using molar ratio of 1:5 (vernonia oil : diamine) without solvent. Highest yields of diamide were obtained using molar ratio of 1:3 (vernonia oil : diamine) at refluxing temperature (61°C) of chloroform. Reactions with molar ratios 1:1; 1:2 and 1:3 (vernonia oil : diamine) carried in the absence of solvent at room temperatures and between 50-70°C did not go to completion due to the formation of solid products, while those of molar ratios 1:5 and 1:7 went to completion between 2-5 hours only when carried at temperatures between 50-70°C. Using the solvents, reactions carried at temperatures 50-70°C with molar ratios 1:3 and 1:5 went to completion between 10-15 hours. Reaction with molar ratios 1:7 went to completion within 4 hours. Use of excess diamine above the reaction ratio of 1:5 (vernonia oil : diamine) at temperatures of 50-70°C resulted to low yield of both aminoamide and diamide. Reactions at higher temperatures yielded rubber-like products. Chloroform and tetrahydrofuran were found to be the best solvent for the preparation of aminoamides and diamides of vernolic acid from vernonia oil. Ethanol was ruled out as a solvent due to the formation of ethyl vernolate via transesterification reaction which competes with a slow amidation reaction.

Standard products were prepared for comparison from pure methyl vernolate. Gas chromatographic (GC) and High Pressure Liquid Chromatographic (HPLC) methods were developed and used for detecting aminoamides and diamides, respectively. The identity and purity of the aminoamide and diamide were assessed by thin-layer chromatography and confirmed by elemental analysis, infrared (IR) and nuclear magnetic resonance (NMR) spectroscopic techniques.

Chapter 1

1.0 INTRODUCTION

Vernonia plant grows as a weed in America, Africa, Madagascar and Asia. It is an annual herb occurring as cultivation weed or in woodlands of E. Zimbabwe, S. Malawi, N. Mozambique, Senegal, Ethiopia and throughout western tropical Africa. The plant can grow from 20 cm to 5 m tall with few branches at the base. When there is enough moisture, the lateral branches rise to secondary branches. It has blue corolla flower head of about 3-4 cm wide and the seeds are dark brown to black with silky hairs (Fig. 1). The plant has been reported to be resistant to diseases and insects and can tolerate extremely hot conditions so long as the moisture is available; hence, it can be grown through irrigation in arid and semi-arid land. Duration times from germination to harvest vary in different areas of Africa and ranges from 4 to 7 months (Perdue et al, 1986).

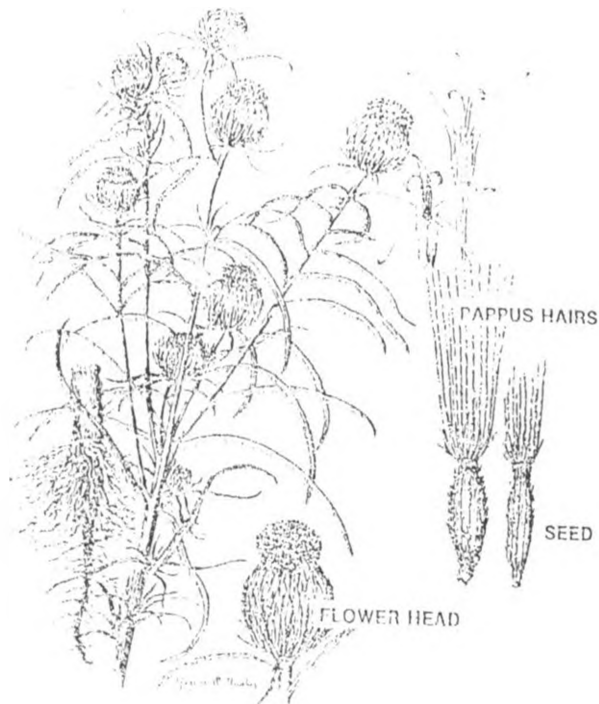


Fig.1 *Vernonia galamensis*

The seed of *Vernonia galamensis* contains about 40% oil of the dry seed weight. Oil contains 72-80% vernolic acid (cis-12,13-epoxyoctadec-cis-9-enoic acid) (Fig. 2), making this plant an excellent source of naturally epoxidized triglyceride oil.

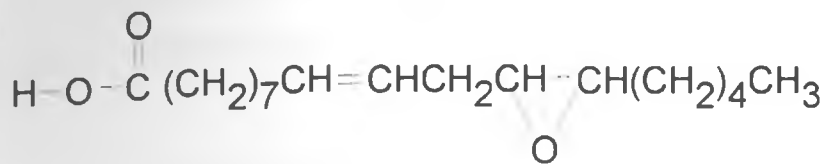


Fig. 2: *Vernolic acid*

Trivernolin is a triglyceride of vernolic acid (Fig. 3).

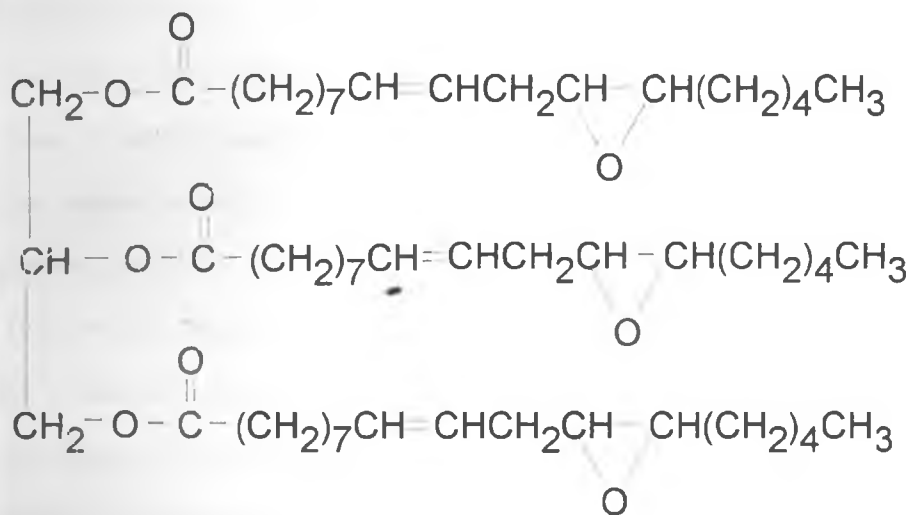


Fig. 3 *Trivernolin*

The unique characteristics of vernonia oil makes it possible for various derivatives to be selectively synthesised under the appropriate conditions. There are three main functional groups within the vernolic acid chain and the following are some of the possible reactions which may take place at each functional group:

- i) Ester group (hydrolysis; aminolysis; transesterification; reduction).
- ii) Double bond (isomerization; halogenation; ozonolysis; epoxidation hydrogenation; polymerization; hydration).
- iii) Epoxy group (hydration; alcoholysis; amination; phosphorylation polymerization).

Unlike terminal epoxy group which is very reactive, the epoxy group in vernolic acid is less reactive under mild conditions due to steric hindrance. Under basic conditions and low temperatures epoxy group in vernolic acid is not reactive.

The vernonia oil being naturally epoxidized would find its use in plastics and coating products (Perdue., 1989). Epoxidized oils (mainly epoxidized soybean oil) serve as plasticisers (for flexibility), stabilisers (to inactive agents in plastics that otherwise cause them to degrade) and generally as highly reactive sites where one triglyceride molecule can become attached to adjacent molecules and these to others, to form interlocking polymer networks. Epoxy oils of greatest value are those with higher oxirane contents. This favours vernolic acid with naturally occurring double bond (-CH=CH-) which can be chemically epoxidized to a product of even higher oxirane content. Further epoxidation of *Vernonia galamensis* oil requires perhaps half the amount of expensive peracid to produce a product with oxirane ratings in the range of fully epoxidized soybean or linseed oils (Carlson and Chang., 1985). Table I shows the oxirane values for *Vernonia anthelmintica* oil, trivernolin, the corresponding epoxidized materials and commercial epoxidized linseed and soybean oils (Krewson et al., 1966).

Table 1. Oxirane oxygen values for *Vernonia anthemintica* oil and other products

	Percentage
Vernonia oil (crude)	3.71
Vernonia oil (refined)	4.26
Vernonia oil epoxidized (crude)	7.13
Vernonia oil epoxidized (refined)	7.35
Trivernolin (97.8%) pure	5.06
Trivernolin epoxidized (crude)	8.30
Trivernolin epoxidized (refined)	8.35
Epoxidized linseed oil	9.00
Epoxidized soybean oil	9.00

1.1 *Vernonia galamensis* Plant In Kenya

Vernonia galamensis is a very common weed in many parts of Kenya. According to recent report (Perdue et al., 1986), Kenya has ideal geographic location and climatic conditions favorable to the development of the *Vernonia galamensis* oil crop; hence, there does not appear to be any reason why *Vernonia galamensis* cannot be grown in large quantity in Kenya. In Kenya, land is available, crop production costs are relatively low and the subsequent processing costs and prices for fats and oils have not increased at the same rate as those for petrochemicals. It is therefore time to evaluate the relationship of fats and oils as renewable resources relative to petrochemicals as non-renewable resources. In fact, successful trials of growing vernonia seed at Kericho in Kenya were made as early as 1975. Moreover, a great deal of research work carried out in USA on vernonia oil has been done using seeds from Kenya (Carlson et al., 1981). Currently, trials on fifty three accessions are being characterized in Kibwezi (Dry land field station of Nairobi University) and Muguga Genebank Field (situated in an area of high agricultural potential) led by Prof. J. Chweya of the University of Nairobi.

Table 2. Direct importation of vegetable oils into Kenya for the period (1983-1987)*(x 10³)Ksh.

	1983	1984	1985	1986	1987
Soyabean oil	26.635	19.775	21.006	69.221	4.611
Ground nuts, olive rape, Colza and Sesame oils	120	5,815	12,473	69,673	7,823
Linseed oil	980	3,324	1,587	1,489	1,781
Palm oil	34	8	202	338	1,766
Palm kernel oil	24	7,692	-	14	68
Castor oil	334	521	290	568	760
Gross vegetable oil importations	874,700	624,794	844,823	789,404	746,690

*Annual Trade Reports, 1983-1987, published by: customs & Excise Dept., Ministry of Finance, Kenya

Ksh. = Kenya shilling.

Vernonia galamensis is highly recommended as a potential new oil crop to be developed in Kenya.

Kenya being an agricultural country that cultivates oil crops such as soybean, linseed, palm, castor, etc, has remained a net importer of vegetable and industrial oils as shown in Table 2. This very unfavorable economic situation calls for a very serious re-evaluation of our national development programme on oil crops in this country.

Vernonia galamensis, which is a good source of naturally occurring epoxidized oil with diverse potential industrial applications, should definitely be incorporated in the Government's development program on vegetable oils.

1.2 *Vernonia galamensis* Plant in Israel

Since 1990, David Mills has been carrying out research in Israel on a number of promising accession and genetic improvement. Some *Vernonia galamensis* seeds used in this research were supplied by him and were either grown in the greenhouse or field. A great deal of research work carried out in Grinberg's laboratory in the Negev, Israel, on vernonia oil was also done using seeds grown by Mills (Grinberg et al., 1994).

At present the Israeli plastics and organic chemicals use epoxidized oil based on soybean. Almost half of epoxidized oil is imported from abroad. Growing vernonia and producing vernonia-based epoxy oil in Israel would save about \$600,000 per year in imports of epoxy oil from abroad and would double the local production of epoxy oil (progress Report 1990-91). Mills reported the possibility of growing *Vernonia galamensis* through irrigation in the Western Negev and Bet-Shean valley.

1.3 LITERATURE REVIEW

Although epoxidized soybean and linseed oils have for many years been widely used for various application, vernonia oil has not been extensively used. It is a potentially new industrial oil which is bound to find wide application.

1.3.1 *Vernonia galamensis*

In 1954, Vernolic acid in seed oil of *Vernonia anthemintica* which contained naturally occurring epoxy ring and a double bond was discovered at the US Department of Agriculture by the finding of Gunstone (Gunstone et al., 1954). Research on vernonia species was intensified and among the recently identified species found to contain vernonia oil is *Vernonia galamensis*. The oil has been analyzed and proved to contain mainly a triglyceride of vernolic acid. Each vernolic acid contains one double bond and one epoxy group on its skeleton (Fig.3). Chemically, the oil has similar or close properties to synthetically epoxidized vegetable oils. In December 1966, C.E. Smith found the seed from Kenya highland to contain 29.9% oil (crude) and vernolic acid yield

was up to 78.2% of the oil. Extraction of the vernonia oil from seeds collected from various parts of Kenya (Nairobi, Kiambu and Embu) has also been carried out in the Department of Chemistry of the University of Nairobi (Kivuti C.R., 1991; Wawira M. A., 1991 and Kimwomi R.K., 1993). From these studies, the seeds were found to contain 29-39% oil (crude). Another study done on the species from arid area of Eastern Ethiopia, (Perdue., 1964) contained 41.9% oil with 72.6% vernolic acid. Other research on *Vernonia galamensis* oil has been done on seeds from different countries in Africa, especially from Ethiopia, Somalia, Sudan, Tanzania, Uganda, Gambia, Mozambique and Zimbabwe.

Greater depth of studies of these species was done by Gilbert (Gilbert., 1986). From his study, it is clear that these species are highly diverse and their center of diversity is in East Africa. In 1975, *Vernonia galamensis* was grown in Kericho, Kenya and found to do quite well.

1.3.2 Vernonia Oil

Vernonia galamensis is an excellent source of epoxy acid-containing triglyceride oil. The seed contains 40-42% oil of which 72-78% is the triglyceride of vernolic acid (cis-12, 13-epoxy-cis-9-octadecenoic acid) (Carlson and Chang., 1985). Other acids found in Vernonia oil include oleic acid (4%), Linoleic acid (13%), Stearic acid (4%), Palmitic acid (4%)

Vernonia galamensis oil is naturally epoxidized (oxirane no. 4.1). It is a clear liquid with low viscosity of only 300 cps at 50°F (Kivuti C.R., 1991). The oil is soluble in organic solvents and paints. Further epoxidation of the double bond of trivernolin raises the oxirane number from 4.1 to 8.1 which is between that of epoxidised linseed oil and epoxidized soybean oil at a reduced cost (Carlson and Chang., 1985).

1.4. Industrial Applications Of Vernonia Oil

Due to its unique chemical structure which is highly and almost equally unsaturated and epoxidized, vernonia oil has several advantages over other epoxidized vegetable oils produced industrially and appears to be a very attractive raw material for various industrial applications.

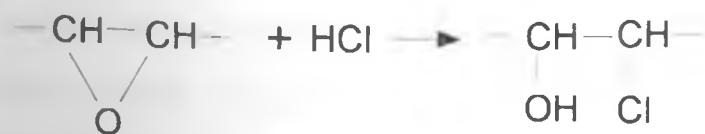
1.4.1 Plasticizers

Refined epoxidized oil and epoxidized trivernolin have been found to have potential value as primary plasticizers for PVC, and they increase heat and light stability (Perdue et al., 1986 and Riser et al., 1962)

PVC is inherently brittle and it easily undergoes photodegradation upon exposure to light or heating. PVC macromolecules gives off hydrochloride molecules as a primary product which is degradation auto-accelerator



Seventy-five to eight-five percent of the manufactured epoxidized soybean oil is used for PVC plastification and stabilization. Epoxidized soybean oil decreases its glass transition temperature and makes it tractable and tough. The epoxy groups of the epoxidized soybean oil react with hydrochloride, prevent the auto-acceleration of PVC degradation and act as its stabilizer.



Vernonia oil has a molecular structure similar to that of epoxidized soybean oil and initial results (Riser et al., 1966) have shown that vernonia oil is an excellent PVC

plasticizer and stabilizer. Since vernonia oil is naturally epoxidized, it is expected to be less expensive than epoxidized soybean oil, it will also be able to substitute the epoxidized soybean oil for PVC modification and capture this large market.

1.4.2 Epoxy coatings

Film forming characteristics were evaluated by spreading the vernonia oil on steel panels, which were then baked in an oven. Coated panels were evaluated for hardness, elongation, and deformation and resistance of the coatings to alkali, acid and solvent. The oil proved suitable for baked films or coatings with excellent physical properties (Carlson et al., 1981). From this research, it was clear that *Vernonia galamensis* oil is a potential raw material for the coatings industry which would phase out volatile organic compounds in some applications such as use of mineral spirits, toluene and xylene in coating industries (Dirlikov et al., 1989 ; Dirlikov et al., 1990 and Muturi, P. M. et al., 1993, Muturi, P.M et al., 1994). These volatile organic compounds (VOC) are ecologically hazardous and their use is being restricted in the USA and other places.

1.4.3 Dibasic acid

A series of dibasic acids have been prepared from vernonia oil e.g. azelaic acid and suberic acid (Ayorinde et al., 1988A) and dodecanedioic acid (Ayorinde et al., 1989A). These dibasic acids and their derivatives which are used in the manufacture of polyurethanes, polyamides, alkyd resins, plasticizers and elastomers are obtained primarily from petroleum.

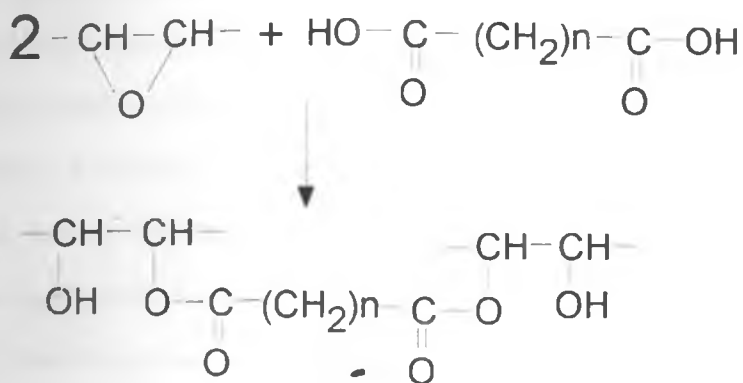
1.4.4 Drying oils

These are substances that react with the oxygen from air to form hard and insoluble resinous material. Drying oils are mostly naturally occurring fatty oils, such as linseed, tung and safflower oils, that consist chiefly of unsaturated triglycerides (Grummit et al., 1965). The oxidation causes polymerization, which is the fundamental reaction of the drying process. Since reactivity with oxygen is due to carbon-carbon double bonds, the

degree of unsaturation of the drying oils is very important. Vernonia oil, which is essentially trivernolin with three double bonds is a good drying oil.

1.4.5. Tough plastics and reinforced elastomers

These involved making either the polyurethane or polyester of castor oil, both of which are soft elastomers. These polymers were combined with cross linked polystyrene to form an interpenetrating polymer network (IPN) (Sperling et al., 1978). IPN may be defined as a combination of two polymers in network form with at least one of the polymer polymerized and/or cross-linked in the immediate presence of the other.



Beginning in 1979, research on different oils was undertaken particularly, epoxy bearing oils from vernonia as well as chemically epoxidized linseed, castor oil, etc. They were found to make useful elastomeric materials.

The reaction of a dibasic acid (Sebacic acid) and an epoxy bearing oil is a typical example of reinforced elastomers. The free hydroxyl group formed in the product is a possible locus of further reactions

In 1989, Ayorinde and his co-workers successfully prepared toughened elastomer from vernonia oil. In 1983 and 1993, Sperling and his co-workers also prepared toughened elastomers from vernonia oil and castor oil. These results demonstrate the potential utilization of vernonia oil for the synthesis of toughened elastomers. Successful synthesis of cross-linked polymer by reacting vernonia oil, *m*-phenylenediamine and stearic acid has been reported. (Grinberg et al., 1994).

1.5 By-Products Of Vernonia Seed And Their Potential Applications

Vernonia galamensis cake (residue after oil extraction) contained 42.5% crude protein, 10.9% crude fibre and 9.5% ash. *Vernonia galamensis* cake has higher levels of lysine, methionine and phenylalanine than *Vernonia anthelmintica*; hence suggesting that it has a better amino acid balance (Carlson et al., 1981). Due to the relatively high level of lysine, methionine and phenylalanine, the vernonia cake seems to be suited for making chicken and other livestock feeds. Feeding experiments are yet to be done.

1.6 Epoxy Containing Fatty Amides

Naturally occurring triglycerides are converted to fatty amides by direct amination under mild conditions. Fatty amides are compounds that exhibit low reactivity and high thermal stability. In industry, the mono-substituted amides (RCONHR) are typically produced from purified fatty acids and primary amines at temperatures of 140-170°C. They can also be prepared under moderate pressure and temperature /or from a reaction of fatty acyl chloride and an amine or fatty ester with amine using sodium methoxide as catalyst (Jordan, E.F., 1961). Stearic acid, 1,2-diaminoethane and methyl stearate react for six hours at 180°C under Nitrogen to give 56% N,N'-ethylene bis stearamide. These methods involve the use of hazardous materials. In 1993, Kent and his co-workers reported the synthesis of novel epoxy-containing fatty amides from vernonia oil by reactions of primary amines with the naturally occurring epoxy triglycerides under mild conditions which does not involve hazardous materials. In the same year, Bilyk prepared ricinoleamide from castor oil without solvent. Another study on direct amidation done by Grinberg and her co-workers in Israel 1994 shows successful synthesis of N,N'-ethylenebis(vernolamide) in the absence of a catalyst at 80°C. Alkanolamides, diamides and aralkylamides have been prepared using tallow oil which is a triglyceride at low temperature of 50°C -60°C. This is approximately 100° lower than present conventional practice employed for the synthesis of fatty amides (Bilyk A. et al., 1992 and 1994). Fatty amides are important chemical intermediate for commerce with applications in

paper coatings, printing, ink additives, slip and anti-block additives for polyethylene films. Basic diamides have a wide variety of applications such as detergent additives, fungicides, rust inhibitors, and corrosion inhibitors, antistatic agents, water repellants, etc (Watanabe S. et al., 1993).

1.7. Objectives

This study reports on the possibilities of using *Vernonia galamensis* seed oil as a source of epoxy containing fatty amides and fatty diamides by direct amidation of the oil. In this present study, a discussion of the isolation and spectroscopic characterization of these fatty epoxidized secondary diamides is reported. Conditions such as time, molar ratio, solvent, temperature are reported

The main objectives for this study are:

- I). Extraction and characterization of the vernonia oil from *Vernonia galamensis* seeds collected from Kenya and Israel.
- ii). Study of the reaction of vernonia oil with aliphatic diamines.

CHAPTER 2

EXPERIMENTAL

2.1 Materials and Equipment

Some of the *Vernonia galamensis* seeds used in this study were grown in Israel in the field or green house, while others were collected in Kenya from Nairobi area. Vernonia oil was obtained either by Soxhlet or room temperature extraction. Some of the crude vernonia oil which were used for the synthesis of fatty amides and fatty diamides in this study was obtained from Ver-Tech, Inc. 11000 Wayeroff Way North Bethesda, USA. All the solvents used were analytical grade purchased from Frutarom Ltd; 1,2-diaminoethane was purchased from Aldrich Chemical Co.; while 1,3-diaminopropane, 1,4-diaminobutane and 1,6-diaminohexane were purchased from Fluka Chemie AGCH-9470 Buchs. Anhydrous sodium sulfate, sodium hydroxide, silica gel for column and (TLC) plates were purchased from E. Merck. All other chemicals were purchased from Aldrich Chemical Co.

Thin-layer chromatographic analyses (TLC) were performed on commercial pre-coated plates (0.25 mm silica gel 60F 254). Visualization were by iodine fumes. Column chromatography was run on silica gel 60 (70-230 mesh; E. Merck) packed in a glass column. Infrared spectra (IR) was carried out in Nujol on a Perkin Elmer 435 V-04 spectrometer. Gas chromatography/mass spectrometry (GC/MS) was performed on a Hewlett Packard 5890A Instrument equipped with a capillary column 12.5 m x 0.25 mm i.d. 0.33 μ m film HPI, temperature program from 150 to 300°C, carrier gas helium. Gas chromatography was carried out on a stainless-steel OV-1 column (10 m x 0.25 mm inner dimension) using a Perkin Elmer model Cp 9001. ^1H - and ^{13}C -NMR Spectra were obtained with a Bruker Wp-200sy spectrometer in CHCl_3 solution (d; TMS). High performance liquid chromatography was performed on HPLC 3120 model which consisted of a constant-delivery pump fitted with a septumless injector having a 20 μ l

loop. The separations of fatty diamides were obtained with stainless steel column (4 x 250 mm). UV detector was used to detect the fatty diamides. Mobile phase was pure methanol (HPLC-grade) with a flow rate of 0.5 ml/min.

2.2 Extraction and Characterization of Vernonia Oil.

Extraction of the oil was done with the seeds from Kenya and Israel.

2.2.1 Materials and Equipment

In Kenya, *Vernonia galamensis* seeds were collected from Wangige and Gachie (Kiambu district, central province) and Embu (Eastern province). Most of the *Vernonia galamensis* plants were found growing beside the roads and farms where maize and beans had been cultivated before. In Israel, some of the seeds accession v-004 (var. *galamensis*) were collected from the Sha'ar Ha'Negev, Experimental Station; Other seeds accession V-001 (var. *Ethiopica*) were obtained from plants grown in the greenhouse at Beer-sheva. All these seeds were supplied by Dr. Mills who is working on genetic improvement by breeding in the Negev desert. Seeds were collected and cleaned before tempering.

2.2.2 Seed Tempering

Clean seeds (300 g) were tempered in the laboratory in a conical flask fitted with thermometer and variable speed stirrer by passing steam through them for two hours. seed temperatures was kept at 90-100°C and seed moistures adjusted upward from ambient 5% to 15% during tempering by adding the required amount of water to the tempering units at appropriate times. Tempered seeds were air dried for three days.

For comparison, other seeds were also tempered by oven heating at 90-100°C without adjusting seeds moisture or by placing in a presto pressure cooker containing 500 ml of water and heated to 90-100°C for one hour.

2.2.3 Seed flaking

Tempered seeds were flaked on a Wolf flaking mill with smooth-faced to obtain seeds in powdered form for easy extraction.

2.2.4 Soxhlet Extraction

The ground seeds (251.86 g) were put in a cheesecloth bag and placed in Soxhlet extractor. *n*-Hexane (3 litres) was used to extract the oil at 50-60°C for five hours. The solvent was then removed by rotary evaporator in vacuo at 40°C to obtain 92.96 g (36.91%) crude oil (dark green in colour). Extraction was repeated with more seed samples (251.91 g; 252.32 g) to obtain average weight of 93.023 g giving an average yield of 36.90%. Soxhlet extraction was repeated with other seeds.

2.2.5. Room Temperature Extraction

The ground seeds (200.50 g) were put in a 500 ml-conical flask, 200 ml of *n*-hexane was added, stirred for 2-3 minutes and left to stand for 12 hours. The mixture was then poured into a two litre separatory funnel. Dark green extract (100 ml) was collected. More solvent was used to extract until the extracting solvent became clear. The solvent from extracts was removed by rotary evaporator in vacuo after every cycle and recovered hexane used to initiate another extraction cycle.

Note, where the recovered hexane was not enough for new cycle, fresh hexane was added. For each cycle, 200 ml of hexane was used and after 5-6 cycles, a total of 79.63 g of crude oil was obtained. The extraction was repeated three times with more seed samples (156.25 g, 200 g, 200 g) to obtain an average weight of 74.56 g of crude oil averaging 39.37% (Table 3). Room temperature extraction was repeated with other seeds.

2.2.6. Oil Refining

Crude oil (213.63 g) was mixed with activated charcoal at 5% by weight, stirred at 60°C for one hour and then filtered to give 84.82% of crude oil.

2.2.7. Degumming

Refined oil (172.5 g) was degummed by stirring with distilled water in the ratio 21:1 (oil: water) at 50°C for one hour followed by centrifuging at 2800 rpm for two hours. Gum and oil were separated and the oil dried at 100°C on a rotary evaporator to obtain 80.54% of degummed oil.

2.3. 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 using *n*-hexane: diethyl ether (70:30 v/v). Visualization was by iodine fumes. The fatty acids appeared as orange/brown spots.

2.4. Identification of Epoxides

The oil samples were spotted on commercially pre-coated silica plates. Developing system was petroleum ether (40-60°C) : diethyl ether : acetic acid (75:25: 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) for half an hour.

The plates were then exposed to fumes of ammonia for two minutes. Epoxides appeared as orange spots on a yellow background (Earle., 1970 and Fioriti et al., 1966)

2.5 Free Fatty Acid Determination

The oil (0.1-0.15 g) was weighed into a 50 ml conical flask to which 10 ml of 1:1 v/v of propanol : toluene was added. Two drops of phenolphthalein solution was added and titrated against a standard solution of 0.02 N of sodium hydroxide while stirring until a semi-permanent pink colour appeared. A blank titration was done using only 10 ml of propanol : Toluene solution (Saul Patal, 1968).

2.6 Transesterification

Sodium methoxide (0.28M) was prepared by dissolving 0.644 g of sodium metal into 100 ml of methanol. This solution (20ml) was mixed with 2 g of vernonia oil (access V-001 var. *galamensis*) in a 100 ml round bottomed flask equipped with a reflux condenser and a magnetic stirring bar. The mixture was refluxed gently for 10-15 minutes until all the oil dissolved to form clear solution. The mixture was cooled, transferred into 250 ml-separatory funnel and saturated solution of sodium chloride was added. The mixture was extracted twice with *n*-hexane. The hexane extracts were combined and washed with distilled water to neutral to remove excess base. The hexane layer was then dried over anhydrous sodium sulfate and solvent removed by rotavapor in vacuo to yield 82% of methyl ester (Kent A. et al., 1992). Similar transesterification was carried out with other portions of vernonia oil. GC/MS of the methyl esters was performed which showed methyl vernolate ester as the main product. Results were consistent with those previously reported (Ayorinde and his group, 1988). Mass spectrum (Fig. 4..) also showed that the reaction had taken place.

2.7. Methyl Vernolate

Isolation of the methyl vernolate from the mixture of methyl ester of vernonia oil obtained after transesterification was achieved by silica gel column chromatography. Silica gel (200 g) 60 (70-230 mesh, E. Merck) was packed into a glass column (4 cm x 80 cm) to a height of 60cm (ratio diameter: height 1:15). Methyl ester of vernonia oil (4 g) was slurried with 10 g of silica gel in 20 ml hexane, solvent was removed and the dry mixture of silica gel and methyl ester was added to the top of the packed column. Isolation of the methyl vernolate was accomplished by successive elution with 100% hexane (100 ml), 1% diethyl ether (200 ml), 4% diethyl ether (300 ml) and 10% diethyl ether (500 ml) in hexane. The progress of these fractionations were monitored by TLC. Elution with 10% diethyl ether in hexane was found to contain methyl vernolate ($R_f = 0.52 - 0.61$). The solvent was removed to obtain 2.84 g of pure methyl vernolate. Yield, 71.03%.

IR (Neat cm^{-1}): (Fig.5) 820, 840 (epoxy group); 1735 (ester carbonyl).

GC/MS (Fig. 6), M/z: 310 (M^+); 279 ($\text{M}^+ - \text{CH}_3\text{O}$); 267 ($\text{M}^{++} - \text{CH}_2\text{CH}_2\text{CH}_3$);
236 ($\text{M}^+ - \text{CH}_3\text{COOCH}_3$)

IR (Neat cm^{-1}) spectrum of the vernonia oil: (Fig.7) 820, 840 (epoxy group);
1735 (ester carbonyl).

2.8 Aminolysis of Methyl Vernolate

Methyl vernolate of vernonia oil was reacted with 1,2-diaminoethane; 1,3-diaminopropane; 1,4-diaminobutane and 1,6-diaminohexane to give their corresponding aminoamide of vernolic acid using molar ratio 1:20 (oil : diamine) at 70°C without solvent.

2.8.1 N-(4-aminobutyl) vernolamide

Methyl vernolate (1.05 g, 3.39 mmol) and (5.97 g, 67.8 mmol) 1,4-diaminobutane were placed into 100 ml-round bottomed flask fitted with a refluxing condenser and a magnetic stirrer. Chloroform (2 ml) was added and the reaction allowed to reflux at the refluxing temperature of chloroform for 24 hours. The progress of the reaction was monitored by TLC and the reaction completion was assumed when no esters were present. The reaction mixture was cooled and transferred into 250 ml-separatory funnel, 40 ml-chloroform added and the organic layer washed with distilled water until neutral to remove excess diamine. The organic layer was dried over anhydrous sodium sulfate and solvent removed by rotavapor in vacuo to obtain solid material. The solid material was washed twice with two portions of 10-ml hexane. The product was vacuum-filtered to obtain solid material weighing 0.98g. The product was very soluble in cold methanol. TLC analysis showed one main spot at the base line and a faint spot at $R_f = 0.66-0.70$, using chloroform: ether: acetic acid (60:40:1 v/v/v) as developing system.

A small column was set up to separate the product on silica gel 60 (70-230 mesh, E.

Merck) packed in glass column (1.5 cm diameter by 22 cm length). Solid product (0.63

g) was eluted with chloroform containing increasing amounts of methanol. Progress of these fractionations was followed by TLC. The fraction with 100% methanol was stripped to obtain 463 mg white powder melting point 118-119°C.

IR (Nujol, cm^{-1}); (Fig. 8) 820, 840 (epoxy group); 3300 (NH secondary amide); 1630 (amide I band) and 1530 (amide II band) of the (amide carbonyl).

$^1\text{H-NMR}$ spectrum (Fig 9), ppm(CDCl_3); 2.88-2.89 ($\text{CH}-\text{CH}$); 2.67-2.7 (CH_2NH_2); 3.20-3.22 ($\text{CH}_2\text{NH-CO-}$); 5.36-5.48 ($\text{CH}=\text{CH}$); 5.9 (NH- CH_2).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); (Fig. 10) 56.4-57.1 ($\text{CH}-\text{CH}$); 123.8-132.4 ($\text{CH}=\text{CH}$); 173.0 (-C-N-).

2.8.2. Synthesis of N-(6-aminohexyl) vernolamide

Methyl vernolate (0.91 g, 2.9 mmol) and (6.76 g, 58.31 mmol) 1,6-diaminohexane were reacted together as above to give 0.8311g of crude product. The product was purified through the column as above to obtain 387 mg of white powder with melting point 118-119°C.

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3300 (NH secondary amide); 1630 (amide I band) and 1525 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum, ppm(CDCl_3); 2.88-2.93 ($\text{CH}-\text{CH}$); 2.63-2.6 (CH_2NH_2); 3.1-3.26 (- $\text{CH}_2\text{NH-CO-}$); 5.3-5.6 (- $\text{CH}=\text{CH-}$), 5.4 (-NH- CH_2).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.5-57.1 ($\text{CH}-\text{CH}$); 123.8-132.5 ($\text{CH}=\text{CH}$); 173.0 (-C-N-).

2.8.3 N-(2-aminoethyl)vernolamide

Synthesis of N-(2-aminoethyl)vernolamide was carried out by reacting (1.12 g, 3.62 mmol) methyl vernolate and (4.34 g, 72.4 mmol) 1,2-diaminoethane to give 0.85 g of crude product. The crude product was purified through a column as above to obtain 357 mg of a white powder with a melting point 116-117°C.

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3300 (NH secondary amide); 1638 (amide I band) and 1540 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum ppm (CDCl_3); 2.88-2.93 ($\text{CH}-\text{CH}$); 2.63-2.69 (CH_2NH_2); 3.1-3.26 ($\text{CH}_2\text{NH-CO-}$); 5.3-5.6 ($\text{CH}=\text{CH}$), 5.4 (NH-CH_2).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.4-57.5 ($\text{CH}-\text{CH}$); 123.7-132.8 ($\text{CH}=\text{CH}$); 173.4 (-C-N-).

2.8.4 N-(3-aminopropyl)vernolamide

Synthesis of N-(3-aminopropyl)vernolamide was carried out by reacting (0.99 g, 3.18 mmol) methyl vernolate and (4.71 g, 63.64 mol) 1,3-diaminopropane to give 0.6612 g crude product. The crude product was purified through a column as above to obtain 311mg of a white powder with a melting point 114-115°C.

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3300 (NH secondary amide); 1630 (amide I band) and 1525 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum ppm (CDCl_3); 2.89-2.91 ($\text{CH}-\text{CH}$); 2.75-2.77 (CH_2NH_2); 3.3-3.35 ($\text{CH}_2\text{NH-CO-}$); 5.37-5.49 ($\text{CH}=\text{CH}$); 6.24 (NH-CH_2).

2.8.5 Synthesis of N,N'-hexamethylene bis(vernolamide)

Methyl vernolate (0.62 g, 2 mmol) and (0.70 g, 6 mmol) 1,6-diamino hexane was placed into 100 ml-round bottom flask fitted with a refluxing condenser and a magnetic stirrer. The reaction was allowed to reflux at 70°C for 40 hours. The progress of the reaction was monitored by TLC. The reaction did not go to completion which was indicated by

presence of esters after 40 hrs. The procedure described in the isolation of N-(6-aminohexyl)vernolamide was used to isolate N,N'-hexylene bis (vernolamide) to give 0.33g crude product (solid). The TLC analysis showed two spots. A small column was set up to separate the product on silica gel 60 (70-230 mesh, E. Merck) packed in glass column (1.5 cm diameter by 22 cm length). The solid product (0.33 g) was eluted with chloroform containing increasing amounts of methanol. The progress of these fractionations was followed by TLC. Two fractions were collected and solvent removed to give fraction 1 -(0.081 g, melting point 123-124°C) and fraction 2 -(0.012 g, melting point 118-119°C).

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3300 (NH secondary amide); 1630 (amide I band) and 1530 (amide I band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); 2.88-2.93 ($\text{CH}=\text{CH}$); 3.1-3.26 ($\text{CH}_2\text{-NHCO-}$); 5.3-5.6 ($\text{CH}=\text{CH}$); 5.4 (NH-CH_2).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.5-57.1 ($\text{CH}=\text{CH}$); 123.8-132.5 ($\text{CH}=\text{CH}$); 173.0 ($-\text{C-N-}$).

2.8.6 N,N'-ethylene bis(vernolamide).

N,N'-ethylene bis(vernolamide) was prepared by reacting (1.02 g, 3.27 mmol) methyl vernolate and (0.59 g, 9.82 mmol) 1,3-diaminopropane to give 0.49 g of crude product. The crude product was purified through a column as above to obtain 211 mg of a white powder with a melting point 121-122°C.

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3300 (NH secondary amide);

1635 (amide I band) and 1540 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); 2.81-2.88 ($\text{CH}=\text{CH}$); 3.29-3.32 ($\text{CH}_2\text{-NHCO-}$); 5.3-5.5 ($\text{CH}=\text{CH}$); 6.1 (NH-CH_2).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.5-57.1 ($\text{CH}=\text{CH}$); 124.3-132.3; ($\text{CH}=\text{CH}$); 174.4 ($-\text{C-N-}$).

2.8.7 N,N'-tetramethylene bis(vernolamide)

N,N'-tetramethylene bis(vernolamide) was prepared by reacting (1.15 g, 3.69 mmol) methyl vernolate and (0.97 g, 11.07 mmol) 1,3-diaminopropane to give 0.67 g of crude product. The crude product was purified through a column as above to obtain 311 mg of a white powder with a melting point 122-123°C.

IR (Nujol, cm^{-1}); (Fig. 11) 820, 840 (epoxy group); 3300 (NH secondary amide); 1630 (amide I band) and 1525 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); (Fig 12) 2.90-2.95 ($\text{CH}-\text{CH}$);
3 25-3.28 ($\text{CH}_2-\text{NHCO}-$); 5.34-5.55 ($\text{CH}=\text{CH}$); 5.87 ($\text{NH}-\text{CH}_2$).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); (Fig. 13) 56.5-57.1 ($\text{CH}-\text{CH}$); 123.8-132.5 ($\text{CH}=\text{CH}$); 173.3 (-C-N-).

2.8.8 N,N'-trimethylene bis(vernolamide)

N,N'-trimethylene bis(vernolamide) was prepared by reacting (1.15 g, 3.72 mmol) methyl vernolate and (0.83 g, 11.16 mmol) 1,3-diaminopropane to give 0.56 g of crude product. The crude product was purified through a column as above to obtain 111 mg of a white powder with a melting point 93-95 °C.

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3300 (NH secondary amide); 1620 (amide I band) and 1520 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); 2.9-2.95 ($\text{CH}-\text{CH}$);
3 2-3.37 ($\text{CH}_2-\text{NHCO}-$); 5.3-5.5 ($\text{CH}=\text{CH}$); 6.2 ($\text{NH}-\text{CH}_2$).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.5-57.1 ($\text{CH}-\text{CH}$); 123.7-132.8 ($\text{CH}=\text{CH}$); 174.0 (-C-N-).

2.8.7 N,N'-tetramethylene bis(vernolamide)

N,N'-tetramethylene bis(vernolamide) was prepared by reacting (1.15 g, 3.69 mmol) methyl vernolate and (0.97 g, 11.07 mmol) 1,3-diaminopropane to give 0.67 g of crude product. The crude product was purified through a column as above to obtain 311 mg of a white powder with a melting point 122-123°C.

IR (Nujol, cm^{-1}); (Fig. 11) 820, 840 (epoxy group); 3300 (NH secondary amide);

1630 (amide I band) and 1525 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); (Fig. 12) 2.90-2.95 ($\text{CH}-\text{CH}$);

3.25-3.28 ($\text{CH}_2-\text{NHCO}-$); 5.34-5.55 ($\text{CH}=\text{CH}$); 5.87 ($\text{NH}-\text{CH}_2$).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); (Fig. 13) 56.5-57.1 ($\text{CH}-\text{CH}$); 123.8-132.5

($\text{CH}=\text{CH}$); 173.3 (-C-N-).

2.8.8 N,N'-trimethylene bis(vernolamide)

N,N'-trimethylene bis(vernolamide) was prepared by reacting (1.15 g, 3.72 mmol) methyl vernolate and (0.83 g, 11.16 mmol) 1,3-diaminopropane to give 0.56 g of crude product. The crude product was purified through a column as above to obtain 111 mg of a white powder with a melting point 93-95 °C.

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3300 (NH secondary amide);

1620 (amide I band) and 1520 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); 2.9-2.95 ($\text{CH}-\text{CH}$);

3.2-3.37 ($\text{CH}_2-\text{NHCO}-$); 5.3-5.5 ($\text{CH}=\text{CH}$); 6.2 ($\text{NH}-\text{CH}_2$).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.5-57.1 ($\text{CH}-\text{CH}$); 123.7-132.8

($\text{CH}=\text{CH}$); 174.0 (-C-N-).

2.9 Reaction Of Vernonia Oil With Aliphatic Diamines

The vernonia oil was reacted with different diamines (1,2-diaminoethane; 1,3-diaminopropane; 1,4-diaminobutane and 1,6-diaminohexane) under different conditions to obtain their corresponding aminoamide or diamide.

2.9.6 N-(6-aminohexyl)vernolamide.

Reaction were performed with molar ratio (1:5 vernonia oil : diamine) for the preparation of N-(6-aminohexyl)vernolamide using 2 g (2.16 mmol) of vernonia oil based on a molecular weight of 926 and 0.376 g (3.24 mmol) 1,6-diaminohexane. Chloroform (2 ml) was added and the reaction mixture allowed to reflux at the refluxing temperature of chloroform for 10-11 hours. The completeness of the reaction was assumed when no oil was present in the reaction mixture. The reaction mixture was cooled and transferred into 250 ml-separatory funnel, 60 ml-chloroform added and the organic layer washed with distilled water until neutral to remove all unreacted diamine and glycerol. The organic layer was dried over anhydrous sodium sulfate for 3 hours and solvent removed using rotatory evaporator to afford solid product. The crude product was dissolved in hot ethyl acetate/methanol solution and placed in a refrigerator for 12 hours to obtain crystals (0.36 g) with melting point 120-121°C. The filtrate stripped of the solvent to obtain crude solid product which was recrystallized from hot ethyl acetate at -10°C for 24 hours. The precipitated epoxy aminoamide was then vacuum-filtered to afford white powder. The product was air-dried, melting point (116-118°C) and spectroscopically characterized.

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3320 (NH secondary amide); 1630 (amide I band) and 1530 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); 2.89-2.93 ($\text{CH}=\text{CH}$); 3.29-3.32 ($\text{CH}_2\text{-NH CO-}$); 5.42-5.47($\text{CH}=\text{CH}$), 5.47(NH)

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.3-57.5 ($\text{CH}=\text{CH}$), 123.7-132.8 ($\text{CH}=\text{CH}$); 173 (-C - N -)

2.9.7 N-(4-aminobutyl)vernolamide

Synthesis of N-(4-aminobutyl)vernolamide was carried out by reacting (2.05 g, 2.213 mmol) vernonia oil and (0.97 g, 11.0 mmol) 1,4-diaminobutane to give 0.86 g of aminoamide with melting point 118-119°C.

IR (Nujol, cm^{-1}); (Fig. 14) 820, 840 (epoxy group); 3300 (NH secondary amide); 1630 (amide I band) and 1530 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum ppm (CDCl_3); (Fig. 15) 2.89-2.90 ($\text{CH}=\text{CH}$); 2.69

(CH_2NH_2); 3.21-3.24 ($\text{CH}_2\text{NH-CO-}$); 5.3-5.4 ($\text{CH}=\text{CH}$); 5.8 (NH-CH_2).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); (Fig. 16) 56.4-57.1 ($\text{CH}=\text{CH}$); 123.8-132.5 ($\text{CH}=\text{CH}$);

173.0 (-C-N-).

2.9.8 N-(2-aminoethyl)vernolamide

Synthesis of N-(2-aminoethyl)vernolamide was carried out by reacting (2.01 g, 2.17 mmol) vernonia oil and (0.65 g, 10.85 mmol) 1,2-diaminoethane to give 1.25 g white powder of aminoamide with a melting point 116-117°C.

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3300 (NH secondary amide); 1635 (amide I band) and 1540 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum ppm (CDCl_3); 2.88-2.93 ($\text{CH}=\text{CH}$); 2.63-2.69 (CH_2NH_2); 3.1-

3.26 ($\text{CH}_2\text{NH-CO-}$); 5.3-5.6 ($\text{CH}=\text{CH}$); 5.4 (NH-CH_2).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.4-57.5 ($\text{CH}=\text{CH}$); 123.7-132.8 ($\text{CH}=\text{CH}$);

173.4 (-C-N-).

2.9.9 N-(3-aminopropyl)vernolamide

Synthesis of N-(3-aminopropyl)vernolamide was carried out by reacting (2.01 g, 2.17 mmol) vernonia oil and (0.80 g, 10.85 mmol) 1,3-diaminopropane to give 0.81 g of a white powder of aminoamide with a melting point 107-109°C.

IR (Nujol, cm^{-1}); 820, 840 (epoxy group) 3300 (NH secondary amide);
1630 (amide I band) and 1528 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum ppm (CDCl_3); 2.89-2.91 ($\text{CH}-\text{CH}$); 2.75-2.77 (CH_2NH_2);
3.3-3.35 ($\text{CH}_2\text{NH-CO-}$); 5.37-5.49 ($\text{CH}=\text{CH}$); 6.24 (NH-CH_2).

2.9.1 N,N'-hexylene bis(vernolamide)

Into a 100 ml-round bottomed flask equipped with a reflux condenser and a magnetic stirring bar was placed ~2 g (2.16 mmol) of vernonia oil based on a molecular weight of 926 and 0.376 g (3.24 mmol) 1,6-diamino hexane. Chloroform (2 ml) was added and the reaction mixture refluxed at the refluxing temperature of chloroform for 10-11 hours. The completeness of the reaction was assumed when no oil was present in the reaction mixture. The reaction mixture was cooled and transferred into 250 ml-separatory funnel, 60 ml-chloroform added and the organic layer washed with distilled water until neutral to remove all unreacted diamine and glycerol. The organic layer was dried over anhydrous sodium sulfate for three hours and solvent removed using rotatory evaporator to afford solid product. The crude product was dissolved in hot ethyl acetate/methanol solution and placed in a refrigerator for 24 hours to obtain 0.46 g solid product, melting point (120-121 $^{\circ}\text{C}$).

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3300 (NH secondary amide);
1630 (amide I band) and 1530 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); 2.88-2.93 ($\text{CH}-\text{CH}$);
3.1-3.26 ($\text{CH}_2\text{-NHCO-}$); 5.3-5.6 ($\text{CH}=\text{CH}$), 5.4 (NH-CH_2)

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.5-57.1 ($\text{CH}-\text{CH}$); 123.8-132.5
($\text{CH}=\text{CH}$); 173.0 (-C-N-).

2.9.5 N,N-Ethylene bis(vernolamide)

N,N'-ethylene bis(vernolamide) was prepared by reacting (2.01 g, 2.17 mmol) vernonia oil and (0.39 g, 6.516 mmol) 1,2-diaminoethane to give 0.75 g white powder, melting point 113-114°C. The above procedure was followed.

IR (Nujol, cm^{-1}); 820, 840 (epoxy group); 3300 (NH secondary amide);

1635 (amide I band) and 1540 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); 2.88-2.93 ($\text{CH}-\text{CH}$);

3.3-3.37 ($\text{CH}_2-\text{NHCO}-$); 5.3-5.5 ($\text{CH}=\text{CH}$); 6.1 ($\text{NH}-\text{CH}_2$).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.5-57.1 ($\text{CH}-\text{CH}$); 124.3-132.3

($\text{CH}=\text{CH}$); 174.4 (-C-N-).

2.9.3 N,N-tetramethylene bis(vernolamide)

N,N'-tetramethylene bis(vernolamide) was prepared by reacting (2.06 g, 2.22 mmol) vernonia oil and (0.59 g, 6.65 mmol) 1,4-diaminobutane to give 0.75 g white powder, melting point 121-123°C.

IR (Nujol, cm^{-1}); (Fig. 17) 820, 840 (epoxy group); 3300 (NH secondary amide);

1630 (amide I band) and 1530 (amide II band) of the amide carbonyl.

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); (Fig. 18) 2.90-2.94 ($\text{CH}-\text{CH}$);

3.25-3.28 ($\text{CH}_2-\text{NHCO}-$); 5.31-5.57 ($\text{CH}=\text{CH}$); 5.89 (NH)

$^{13}\text{C-NMR}$ (CDCl_3 ppm); (Fig. 19) 56.5-57.1 ($\text{CH}-\text{CH}$); 123.8-132.5

($\text{CH}=\text{CH}$); 173.3 (-C-N-).

2.9.4 N,N-trimethylene bis(vernolamide)

N,N'-trimethylene bis(vernolamide) was prepared by reacting (2.02 g, 2.18 mmol) vernonia oil and (0.48 g, 6.55 mmol) 1,3-diaminopropane to give 0.68 g white powder melting point 117-119°C.

IR (Nujol, cm^{-1}): 820, 840 (epoxy group); 1630 ~ 1525 (amide carbonyl);
3300 (NH secondary amide).

$^1\text{H-NMR}$ spectrum (CDCl_3 ppm); 2.90-2.95 ($\text{CH}-\text{CH}$);
3.22-3.31 ($\text{CH}_2-\text{NH CO-}$); 5.40-5.52 ($\text{CH}=\text{CH}$); 6.25 (NH).

$^{13}\text{C-NMR}$ (CDCl_3 ppm); 56.3-62.5 ($\text{CH}-\text{CH}$); 123.7-132.8
($\text{CH}=\text{CH}$); 174.0 (-C - N -)

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Oil Extraction And Characterization

The results obtained in the extraction of oil are consistent with previously reported values (Grinberg et al., 1994 and Ayorinde et al., 1990). Extraction by soxhlet with hexane was found to yield more oil (36-40%) in comparison with the one obtained by stirring ground vernonia seeds in hexane at room temperature (20-39%) (see Table 3). Soxhlet extraction was found to be more economical as less solvent was used and the time it took to extract all the oil indicated by the clear appearance of the solvent in upper thimble (upper chamber) is very short. Stirring at room temperature used large amount of solvent. Although much of the solvent was recycled and the work involved to remove solvent at each stage is extensive and time consuming. Seeds collected in Kenya (var. *Nairobensis*) were found to yielded less oil (37%) compared to accession V-001 (var. *Ethiopica*) grown in greenhouse in Israel (39-40%). The method involving lipase deactivation by oven heating of seed without moisture adjustment yielded the oil containing very high amount of carboxylic acids (8.09-8.16%), while pressure cooker heating method and steam lipase deactivation yield the oil containing small amount of carboxylic acid (0.35-0.45%). This shows moisture adjustment is necessary for complete lipase inactivation. The oil extracted at room temperature shows high content of vernolic acid (75%) compared with the one obtained by Soxhlet extraction (71%).

Table 3. Oil Recovery With Soxhlet And Cold Extraction.

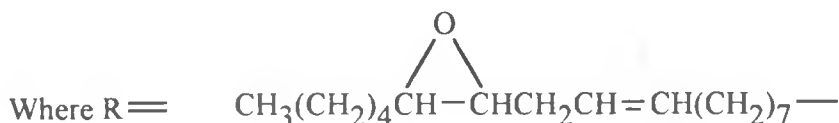
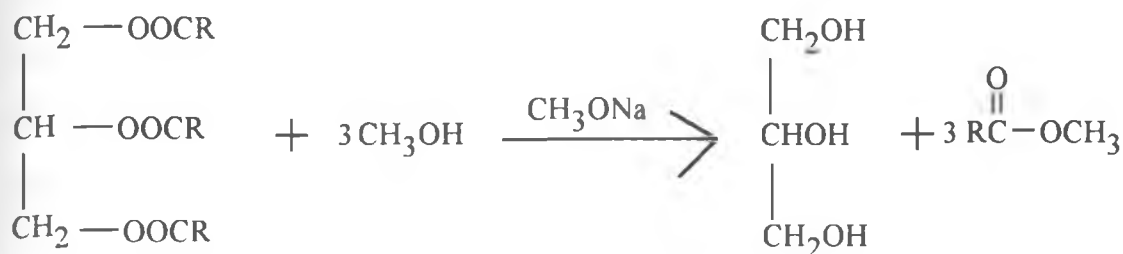
	Soxhlet Extraction	Cold extraction
Dry seeds	Yield (%)	Yield (%)
var. <i>Nairobensis</i>	36.91	-
v-001 (var. <i>Ethiopica</i>)	40.53	39.00
V-004 (var. <i>galamensis</i>)	26.00	20.50

The oil obtained from soxhlet and cold extraction was transesterified to determine the composition of fatty acid in the vernonia oil (Table 4). Methyl esters of vernonia oil were obtained by refluxing the oil with 0.28 M NaOCH₃, cooled, saturated solution of NaCl added and extracted with hexane. The relative weight of the fatty acids in the oil was determined by GC/MS (Fig. 4)

Table 4: Fatty Acid Composition As Methyl Esters by GC/MS

	Soxhlet Extraction		Cold Extraction	
	V-001 <i>Var. Ethiopica</i>	V-004 <i>Var. galamensis</i>	V-001 <i>Var. Ethiopica</i>	V-004 <i>Var. galamensis</i>
Vernolic acid	71	59	75	65.1
18.2	23.59	26	13.91	24.45
18.1	-	-	3.91	-
18.0	-	7.27	1.28	5.79
16.0	4.41	5.96	2.66	4.64
Unidentified material	-	-	2.3	-

The following equation represents the main esterification process of vernonia oil using sodium methoxide/ methanol solution.



(II)

3.2 Aminolysis of Vernonia Oil with Aliphatic Diamines

As mentioned in the introduction, fatty amides are important intermediates and products for the industry. Their applications could be found in paper coatings, printing ink additives slip and anti-block additives for polyethylene films. Fatty bisamides are used primarily to increase slip, reduce blocking, and reduce static in polymeric systems. Other special applications include co-solvents or coupling agents for polyamide resins, fillers for electrical insulation coatings, additives for asphalt to reduce cold flow and synthetic waxes for textile treatments. Bisamides have been used to increase lubricity and are more efficiently than primary amides. N,N'-ethylene bis(vernolamide) was recently prepared by reacting vernonia oil with 1,2-diaminoethane. (Grinberg, et al., 1994) as part of a project of preparing the new chemical derivatives based on vernonia oil. This research focused on a reaction of vernonia oil with aliphatic diamines, including synthesis, isolation and characterization of the intermediate and the final products. Different parameters which influences the reaction in order to find the optimal conditions for the preparation of amides and elucidation of the stages of the reaction are also studied.

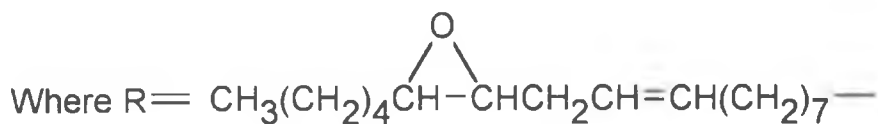
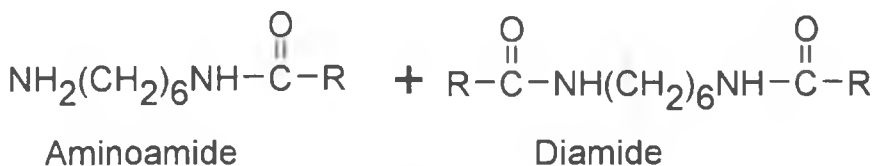
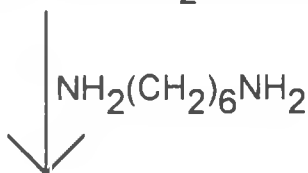
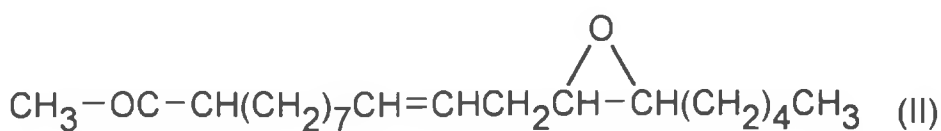
3.2.1 Reaction of Methyl Vernolate with 1,6-Diaminohexane

The initial reaction was an aminolysis of methyl vernolate which would be used as standard for analysis of the vernonia oil amidation products. This was necessary since the oil contains other fatty acids as shown by GC/MS (Fig. 4) analysis of the methyl esters of vernonia oil.

The methyl vernolate (II) was isolated from the mixture of esters of vernonia oil by column chromatography as described above. The IR (Fig. 5) and mass spectra (Fig. 6) analyses were consistent with structure (II). The R_f of methyl vernolate was in the range of 0.52-0.61 as reported (Ayorinde et al., 1990).

A series of reactions of methyl vernolate with 1,6-diaminohexane were carried out at 70°C varying the ester:diamine molar ratios both without the solvent and in CHCl_3 as the solvent. In order to establish the optimum conditions for aminoamide and diamide synthesis from the esters, the extent of aminolysis was monitored by TLC. The reaction of methyl vernolate with excess diamine (1: 20, ester : diamine ratio) took a shorter time to go to completion (24-26 hours). No ester could be found in the reaction mixture after this period. The IR spectra data of the crude solid products obtained after washing with water and *n*-hexane (see procedure in (a)) were all similar. The IR spectral data (Fig. 20) shows no change in the absorbance of the epoxy group ($820, 840 \text{ cm}^{-1}$), but the ester band between $1740 \sim 1730 \text{ cm}^{-1}$ disappeared. This shows that the epoxy group was stable under our working conditions. New absorption bands appeared at $3300, 1645$ and 1550 cm^{-1} indicating that an aminolysis reaction had taken place at the ester bond giving an amide as the products.

Below is an aminolysis reaction of methyl vernolate with 1,6-diaminohexane.



The TLC analysis of the aminolysis product shows two main spots in all cases. Small column was set up to separate the product. Elution with chloroform followed by slowly increasing the amount of methanol yielded two main products (A and B). From $^1\text{H-NMR}$ spectra (Fig. 21 and 22), the two derivatives were identified as aminoamide and diamide of vernolic acid. $^1\text{H-NMR}$ spectrum of product B suggests that it is an aminoamide since it has a free NH_2 group. This is indicated by presence of methylinic protons adjacent to NH_2 group which is observed at 2.6 ppm. $^1\text{H-NMR}$ spectrum of the product A shows no free NH_2 group and was identified as N,N'-hexamethylene bis(vernolamide) (structure iv). N,N'-hexamethylene bis(vernolamide) is soluble in chloroform and partially soluble in other organic solvent such as acetone, methanol, acetonitrile, etc. Aminoamide is soluble in most organic solvents such as methanol, acetone, chloroform, etc.

GC analysis shows one major peak of product B, [N-(6-aminohexylene) vernolamide] (Fig. 23). Under these conditions, product A, [N,N'-hexamethylene bis(vernolamide)] could not be analyzed as peaks were not detected. Diamide, B, was found to absorb at 210 nm in UV region and thus analysis of the diamide was performed with high-performance liquid chromatography (HPLC) using UV detector and methanol as the mobile phase (See Fig. 24).

3.2.2 Reaction of Methyl Vernolate II with Other Aliphatic Diamines

Methyl vernolate (II) was reacted with 1,2-diaminoethane; 1,3-diaminopropane and 1,4-diaminobutane using mainly 1:3, ester : diamine ratio in absence of solvent for the synthesis of diamides and 1:20, ester : diamine ratio in 2 ml-chloroform for the synthesis of aminoamides. IR spectra of all the products were similar in all cases. Pure samples of aminoamide and diamide were obtained by column chromatographic separation method. Identification of the products were determined by TLC, IR, NMR, GC & HPLC. The spectra were very similar to those of corresponding aminoamides and diamides respectively prepared from the reaction of methyl vernolate with 1,6-diaminohexane. Spectra of the aminoamide and diamide (Fig 8-19) prepared from 1,4-diaminobutane are given at the appendix.

3.3. The Reactions of Vernonia Oil with 1,4- Diaminobutane

Reactions of vernonia oil with 1,4-diaminobutane with and without solvents using different molar ratios of vernonia oil to 1,4-diaminobutane were carried out in order to determine the most optimum method to utilize for the synthesis of aminoamide and diamide.

3.3.1. The Effect of Molar Ratio of Vernonia Oil to Diamine without Solvent.

The reactions of vernonia oil with 1,4-diaminobutane were chosen as the model reactions. Investigation was began by first studying the reactions of vernonia oil with different molar ratios of oil to diamine. The reactions were performed at 70°C without solvent. As described before, GC and HPLC separation techniques, using the same

conditions as for the products obtained from the methyl vernolate, were used to follow the products formation (aminoamide and diamide) in the reaction mixture. The products formation and reactants disappearance were also monitored by TLC analysis. The IR analysis was also performed in order to confirm the completion of each reaction (i.e the disappearance of carbonyl at $1740\text{--}1730\text{ cm}^{-1}$ of ester group and the appearance of the carbonyl peak at 1645 cm^{-1} and 1550 cm^{-1} of amide group). The products obtained were identified chromatographically by comparison to standards prepared from methyl vernolate. Samples were withdrawn periodically from the reaction mixture, dissolved in known amount of methanol and analyzed using both GC and HPLC-separation methods.

Table 5 shows the effect of the molar ratios of oil : diamine on the percentage yields of aminoamide and diamide at 70°C in the absence of solvent at different times. The reactions with molar ratios of vernonia oil : diamine (1:1; 1:2; and 1:3) did not go to completion due to the formation of the insoluble products. When the molar ratio was increased to 1:5, the reaction mixture was soluble throughout the reaction period, while the reaction of molar ratio of 1:7 was homogeneous throughout the reaction period.

Table 5: Effect of molar ratio between the oil and 1,4-diaminobutane on the yield of aminoamide and diamide at 70°C in the absence of solvent at different times:

Molar Ratio	0.5 hour		1 hour		2 hour	
	aminoamide	diamide	aminoamide	diamide	aminoamide	diamide
1:1	2.38	3.4	1.54	6.3	2.2	13.5
1:2	24.9	4.2	22.2	10.27	31.09	23.42
1:3	28.7	10.2	34.7	17.3	36.62	26.3
1:5	61.22	5.56	72.33	13.37	56.41	20.4
1:7	40.79	7.95	43.84	9.4	42.9	16.48

When the reaction was carried out using molar ratio 1:1 (vernonia oil : diamine), the products (aminoamide and diamide) are very low. The yield of aminoamide does not seem to increase with the increase of the reaction time, while the formation of diamide increases with the increasing of the reaction time. As the concentration of diamine is increased, the formation of aminoamide is also increased. The formation of aminoamide ranged from 2.38% in (1:1) to a maximum of 72.33% in (1:5) ratio for the reaction period of one hour. Longer reaction period lowered the concentration of the aminoamide and increased the formation of diamide. Further increase of the ratio of oil : diamine to 1:7 resulted in the decrease of the formation of aminoamide. The increase of the diamine from an oil : diamine ratio of 1:1 to 1:7 shows an increase of the formation of diamide to the maximum formation at the ratio of oil : diamine of 1:3 at all reaction periods and subsequent decrease in formation of diamide in the reaction ratios of 1:5 and 1:7. It is probable that the use of higher concentration of diamine (ratio of 1:5 and 1:7) lead to the formation of the side product where the epoxy ring is opened by the diamine. This probably accounted for the decrease in the formation of both aminoamide and diamide. It was noted for example, that a rubber-like product was formed when the reaction was carried out with the oil : diamine molar ratio 1:10 at 70°C after one hour. Increasing the reaction time led to the formation of more rubber-like products. Increasing reaction time did not lead to increasing the yield. When the reaction mixture of molar ratio 1:3 was allowed to proceed for about 18 hours, polymeric products were obtained. The reaction with molar ratio 1:5 at 70°C took five hours to completion, while that at molar ratio 1:7 took two hours.

3.3.2 Effect of Temperature on Aminolysis Without Solvent.

Aminolysis of vernonia oil with diamines is affected by both the temperature and the molar ratio of the two reactants. It was observed that the reaction did not go to completion when the molar ratio 1:5 was reacted at 50°C. The reaction mixture solidified within five hours. The IR spectroscopic analysis of the product showed a weak ester band at 1720-1730 cm^{-1} after five hours, confirming that all the oil had not been

converted to amide after five hours. The result of the reaction of molar ratio 1:5 (vernonia oil : 1,4-diaminobutane) performed at room temperature, 50°C and 70°C are presented in figure 25

It is clear from figure 25 that there is no difference in the yield of aminoamide at both 50°C and 70°C. The yield increased with time reaching the maximum after about one and half to two hours and then began to decrease. At room temperature the reaction is slow. The reaction was, however, relatively fast from the beginning to about two hours. There after it increased slowly and did not reach the maximum even after four hours of the reaction.

Fig.25. The effect of temperature on the yield of aminoamide with molar ratio of 1:5 vernonia oil : 1,4-diaminobutane in absence of solvent.

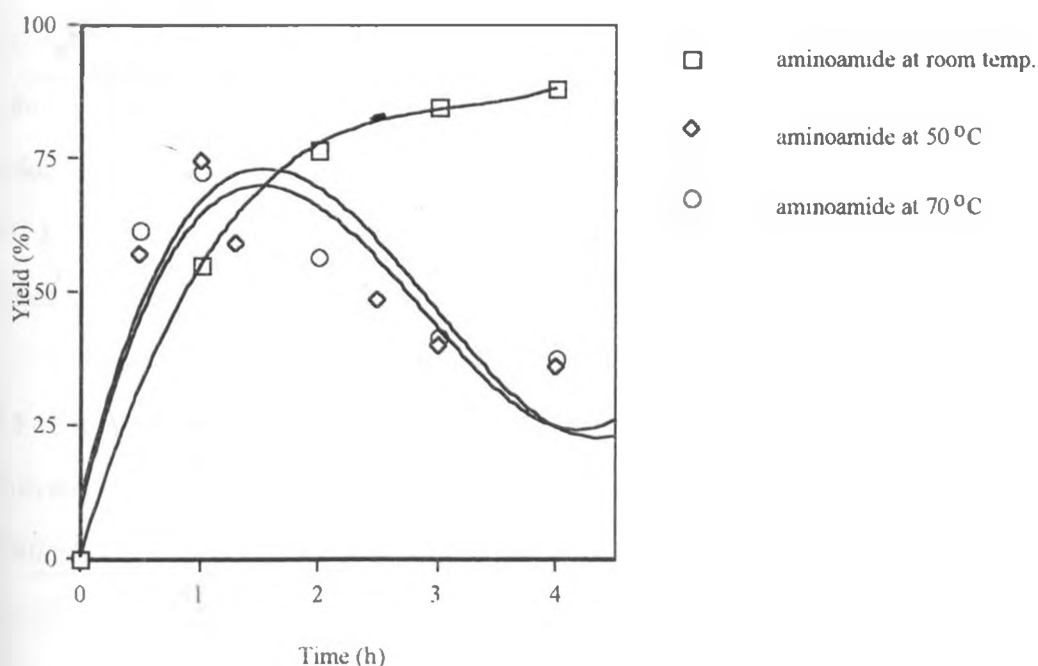


Fig. 26. The effect of the temperature on the yield of diamide in the reaction of the vernonia oil and 1,4-diaminobutane in molar ratio 1:5 (vernonia oil : diamine).

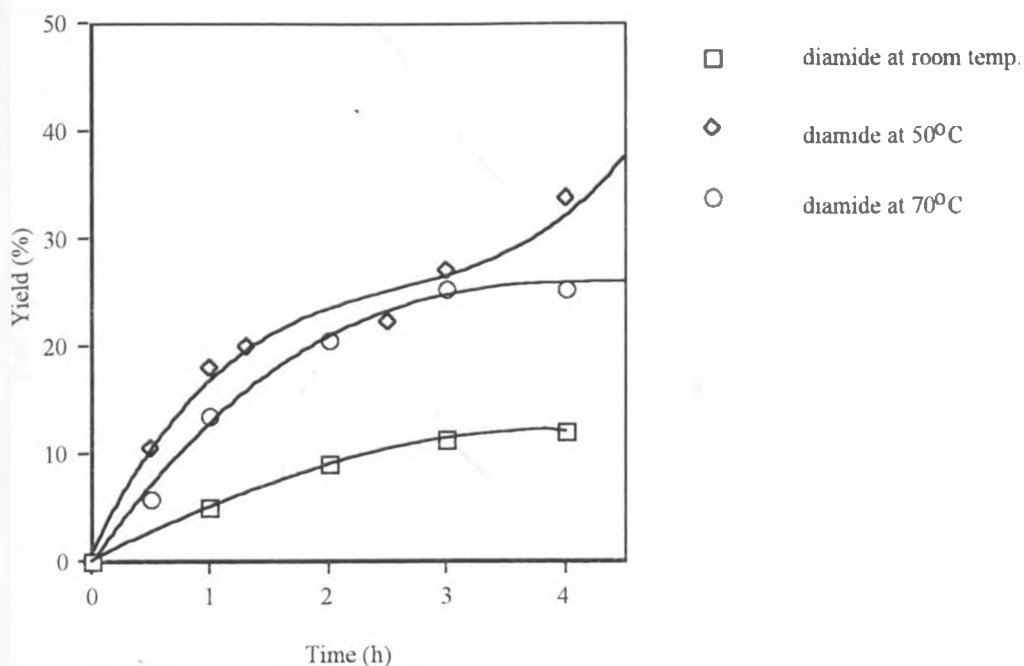


Figure 26 presents the effect of the temperature on the yield of diamide in the reaction of the molar ratio 1:5 (vernonia oil : 1,4-diaminobutane). At room temperature only 10% of diamide is formed after three hours of the reaction. By increasing the temperature to 50°C and 70°C the yield of diamide increased and gave about 25% after three hours of reaction.

3.3.3 Effect of the Amount Solvent on Percentage Yield of Both Derivative

A solvent was added to the reaction mixture in order to overcome the problem of solidification. The reactions with molar ratio 1:3 (vernonia oil : 1,4-diaminobutane) were carried out varying the amount of chloroform.

Fig. 27. The effect of amount of solvent added in a reaction of vernonia oil + 1,4 - diaminobutane (molar ratio of 1:3 vernonia oil : diamine) after 3 hours.

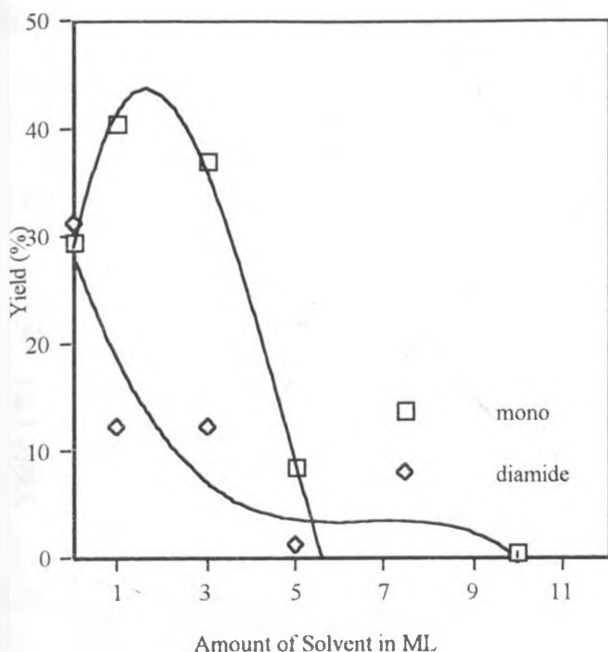


Figure 27 shows the effect of the amount of chloroform added on the yield of both aminoamide and diamide at the refluxing temperature of chloroform after 3 hours. As the amount of chloroform is increased, the yield of aminoamide increases reaching maximum at using 3 ml of solvent and then decrease with the use of more than 3 ml of the solvent. However, in case of diamide, the yield decreases with increase of solvent. This shows that the formation of both product is very much dependent on the concentration of the reactants. This can be explained in terms of dilution factor. The more the dilution, the less the collision of the reactants, thus, less product formation.

3.3.4. The Effect Of Molar Ratio On The Aminolysis Of Vernonia Oil

In 2 ml Chloroform.

Minimum amount of solvent at which the reaction mixture remained homogeneous was chosen and studies conducted varying the amount of diamine in order to investigate the

effect of molar ratio. Reactions with molar ratios 1:1 ; 1:3 and 1:5 of vernonia : diamine were carried out.

Fig.28 The effect of molar ratio on % yield of aminoamide using chloroform as the solvent at refluxing temperature of chloroform (61°C). Molar ratio of 1:3 vernonia oil : 1,4-diaminobutane.

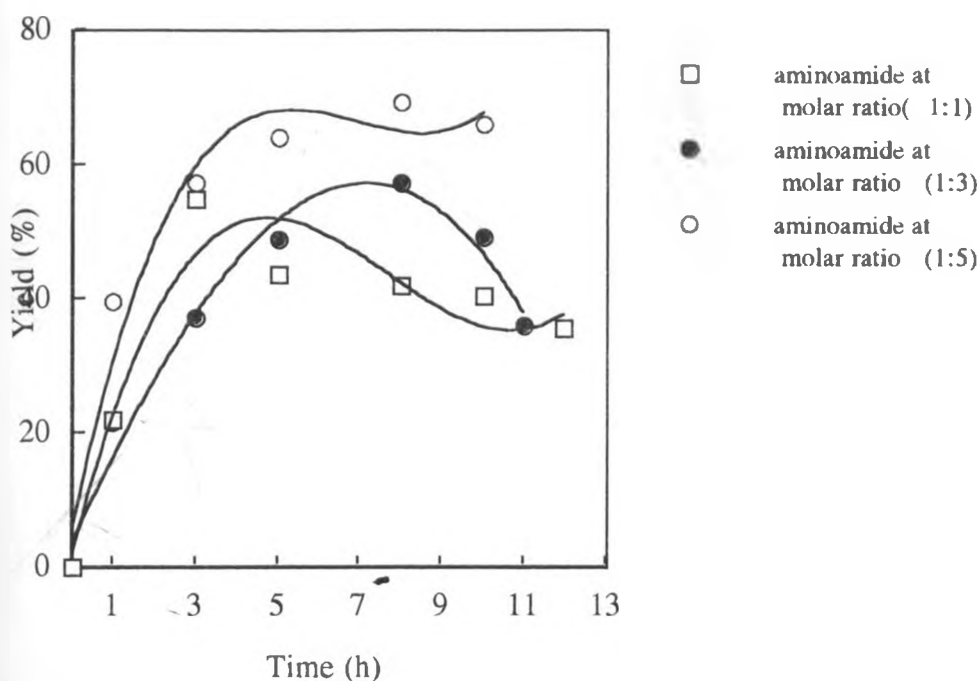


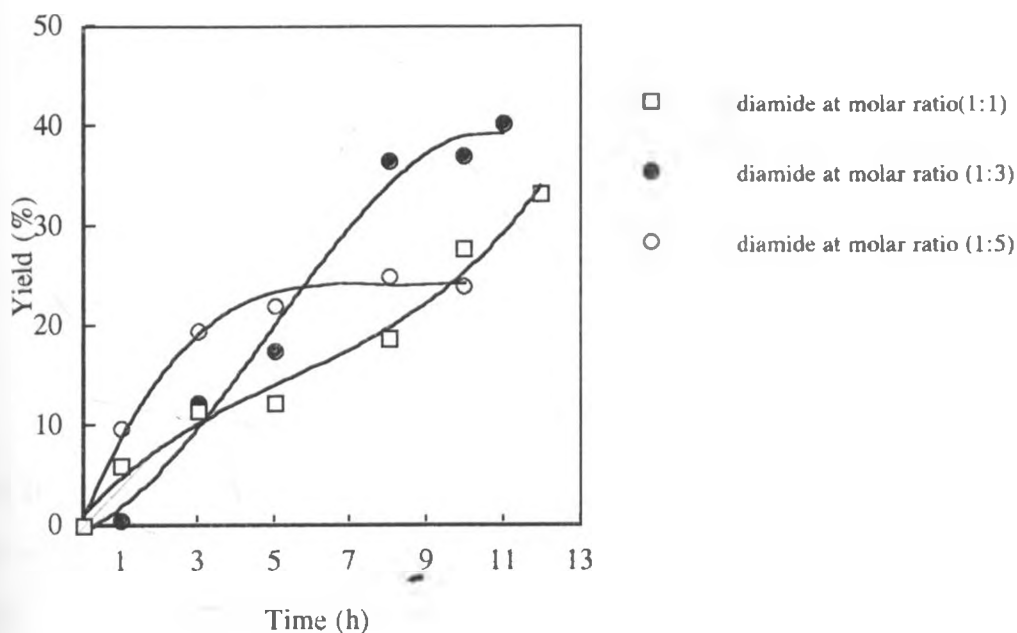
Figure 28 presents the result obtained for the yield of aminoamide in these conditions. The yields of aminoamide increases with molar ratio. The highest yield of aminoamide was obtained from the reaction of molar ratio 1:5 (vernonia oil : diamine). This same molar ratio 1:5 gave the highest yield when the reaction was carried out in the absence of solvent.

Figure 29 shows the effect of molar ratio on the yields of diamide in 2ml chloroform. The yields of diamide increased with molar ratio upto 1:3 (vernonia : diamine). Further increase of diamine results in decrease of diamide. It is clear from figure 29 that to obtain high yield of diamide, less amount of diamine is required; while to obtain high

yield of aminoamide more amount of diamine is required. The results are consistent with those obtained without solvent.

Fig. 29. The effect of molar ratio on % yield of diamide using chloroform as the solvent at refluxing temperature of chloroform (61°C). Molar ratio of 1:3

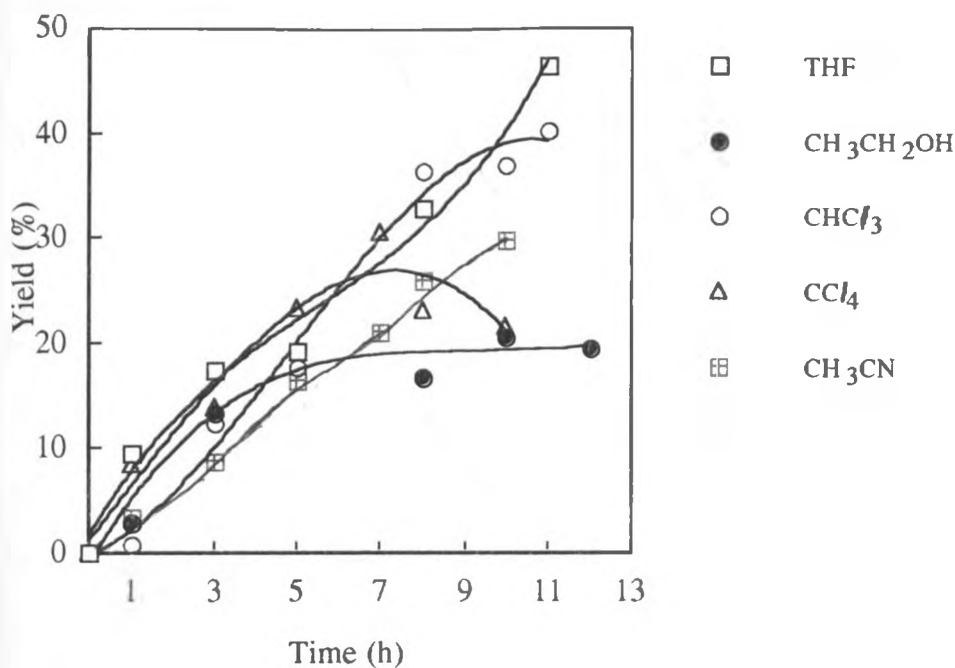
vernonia oil : 1,4-diaminobutane.



3.3.5 The Effect of Different Solvent on the Aminolysis

In order to investigate the effect of different solvents on aminolysis of vernonia oil, reactions were carried out in each solvent (chloroform, ethanol, tetrahydrofuran, acetonitrile and carbon tetrachloride) with molar ratios 1:3 and 1:5 (vernonia oil : diamine), respectively. The results are presented in figures 30 and 31. It is clear from these figures that chloroform is the best solvent to use for preparing both derivative (aminoamide and diamide) from vernonia oil and aliphatic diamine. Lowest yields were obtained from the reactions performed in carbon tetrachloride.

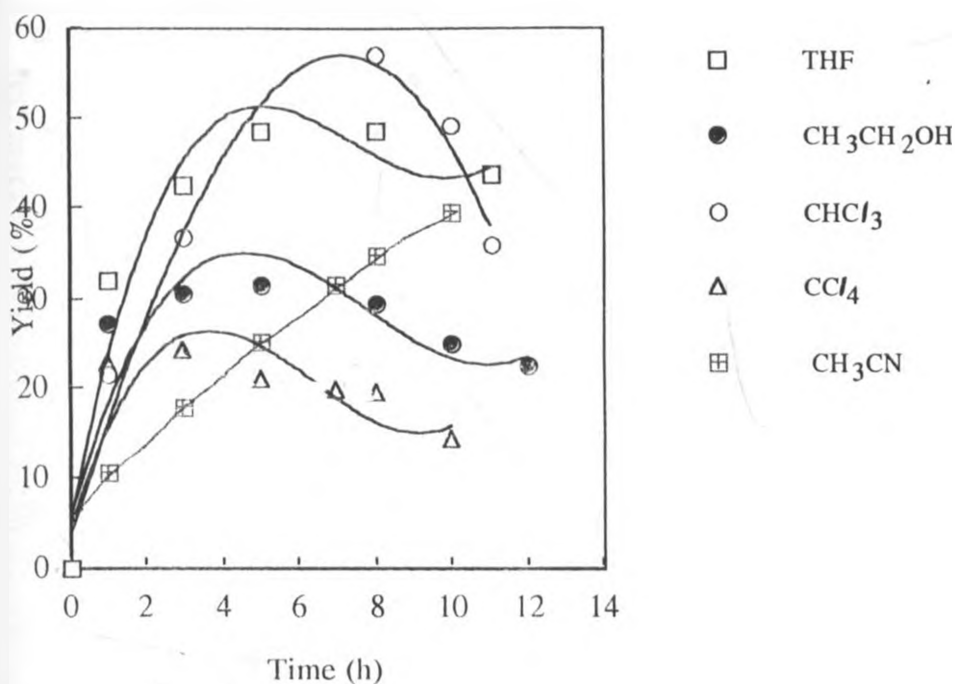
Fig. 30 The effect of different solvent on the % yield of diamide in the reaction of vernonia oil + 1,4-diaminobutane (molar ratio 1:3 vernonia oil : diamine).



The crude product obtained from the reaction mixture with carbon tetrachloride was rubber-like. This indicates that other side reactions might have occurred. Probably epoxy ring opened and gave rise to polymeric product. It was expected that more polar solvent would be the best since the reaction is nucleophilic. Polar solvent should stabilize the transition state, thus, lowering the activation energy which leads to high yield. It is, however, noted that the yield of both product is lower in ethanol than in chloroform.

The crude product obtained from the reaction in ethanol was analyzed using GC/MS. The spectra showed the presence of ethyl esters. Another reaction with molar ratio of 1.0.5 (vernonia oil : 1,4-diaminobutane) refluxed for 12 hours showed similar spectra. This clearly demonstrated that transesterification reaction competed with aminolysis as side reaction giving rise to ethyl esters which are less reactive than triglyceride oils. This accounts for the low yield of both products.

Fig. 31 The effect of different solvent on the % yield of aminoamide in the reaction of vernonia oil + 1,4-diaminobutane (molar ratio 1:3 vernonia oil : diamine).

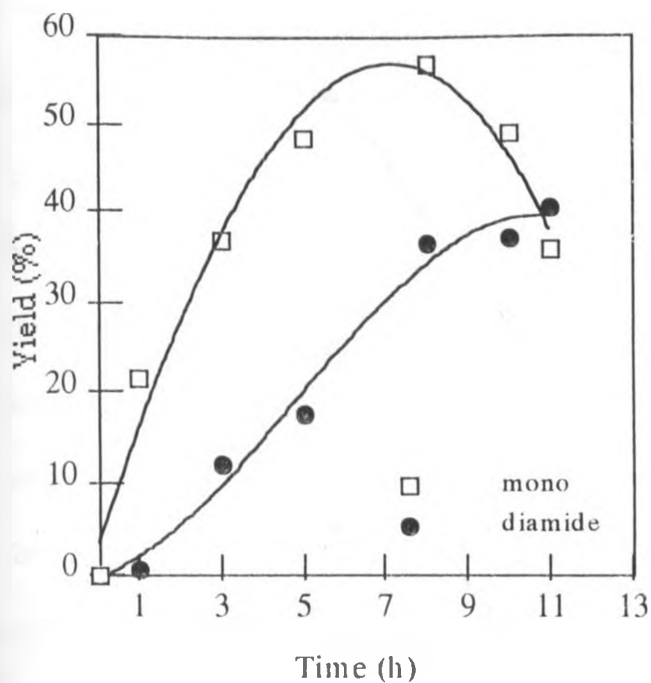


A blank reaction set up without diamine, i.e. vernonia oil in 2 ml-ethanol refluxed for 12 hours showed no reaction. This shows that diamine in presence of alcohol acts as a catalyst for transesterification of the oil. The use of ethanol as a solvent in aminolysis reactions of triglyceride was therefore ruled out.

3.3.6 The Reaction Profile

After determining that aminoamide and diamide could be prepared by the reacting vernonia oil with diamine, studies were conducted to find out whether the reaction was consecutive. Reaction with vernonia oil and 1,4-diaminobutane in 2 ml-chloroform were conducted using molar ratios 1:3 and 1:5, respectively.

Fig. 32. shows the reaction profile of the yield of aminoamide and diamide in chloroform at the refluxing temperature of chloroform.



As expected, the rate of aminoamide formation is faster at the beginning, reaching maximum after about six hours and then starting to decrease while that of diamide is slow at the beginning and continue increasing with time. The reaction profile shows a characteristic of two step reactions. Similar reactions conducted with other diamine (1,2-diaminoethane; 1,3-diaminopropane and 1,6-diamino hexane) were observed.



Finally, an attempt was made to prepare diamides from vernonia oil and 1,4-diaminobenzene. No product formation was observed. The difference can be explained by the structure of 1,4-diaminobenzene where the non-bonded pair of electrons, responsible for the nucleophilicity of amino groups, is dispersed over the aromatic ring. Aromatic diamine are weaker nucleophile than aliphatic amines.

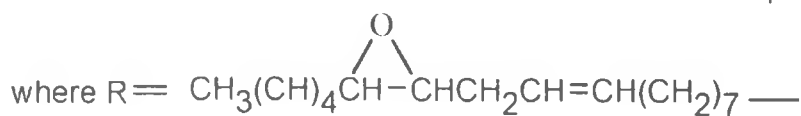
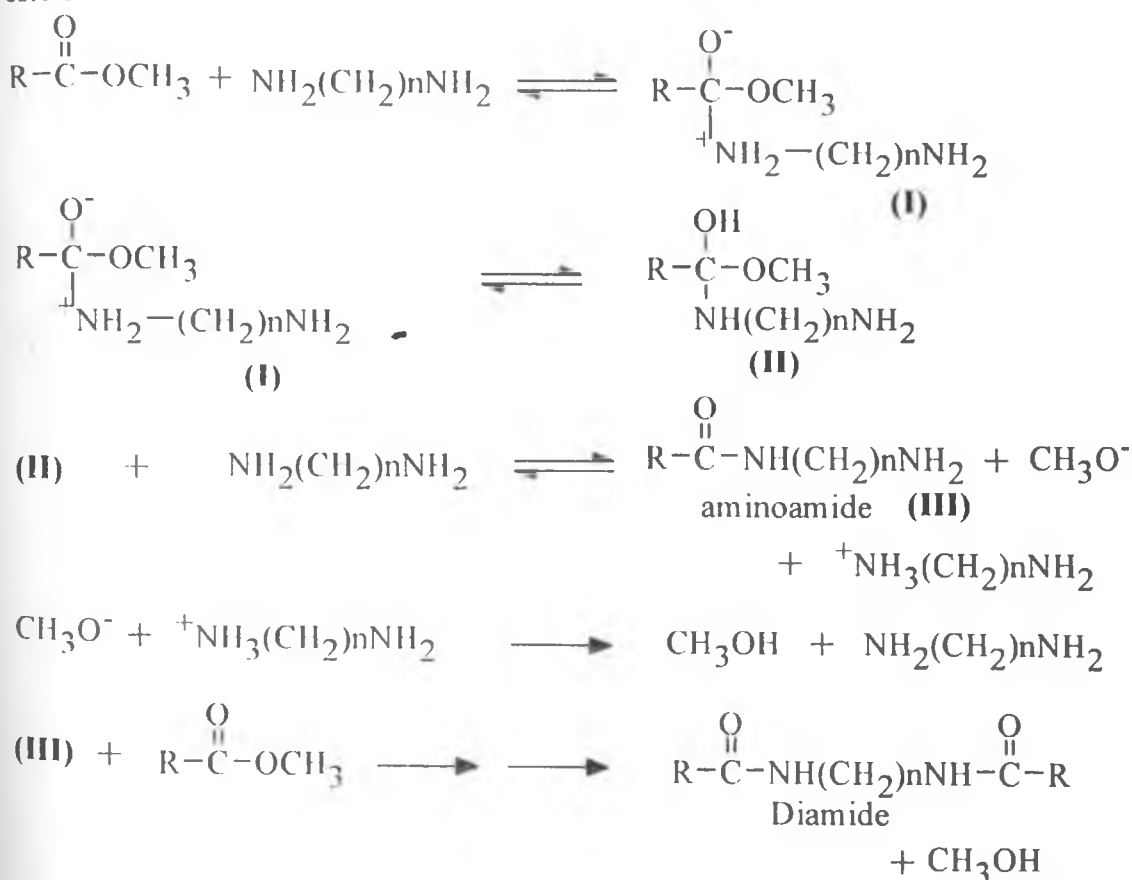
CONCLUSION

It was found that both soxhlet and room temperature stirring extraction methods of vernonia seeds with hexane are effective methods for the extraction of vernonia oil. The soxhlet extraction method was more efficient and convenient than room temperature stirring extraction method; however, the former method produced the oil that contained more free fatty acids and relatively low content of vernolic acids. Autoclave tempering method was confirmed to be the most superior method of lipase deactivation. *Var. Ethiopica* was found to contain the highest yield of vernonia oil and relatively high content of vernolic acid.

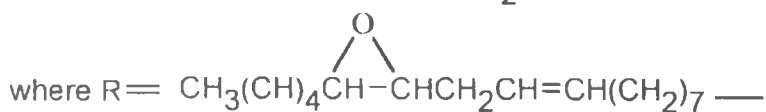
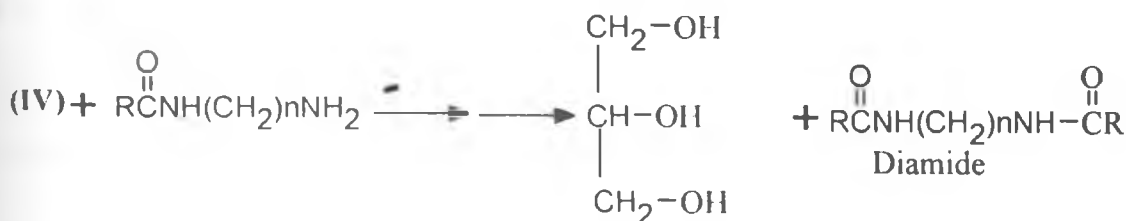
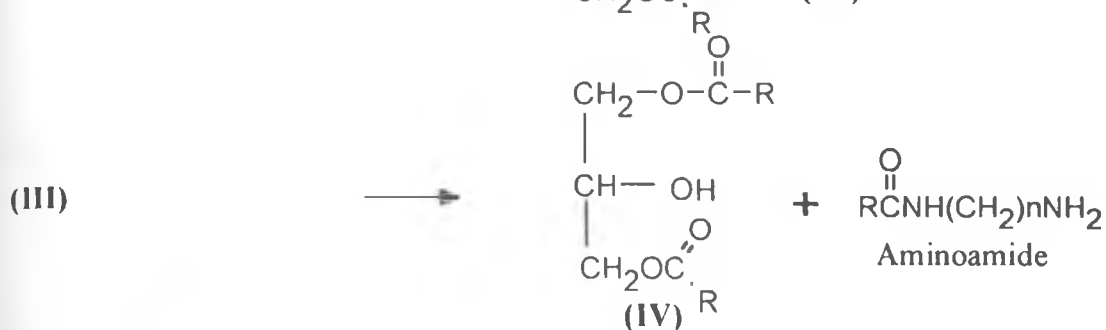
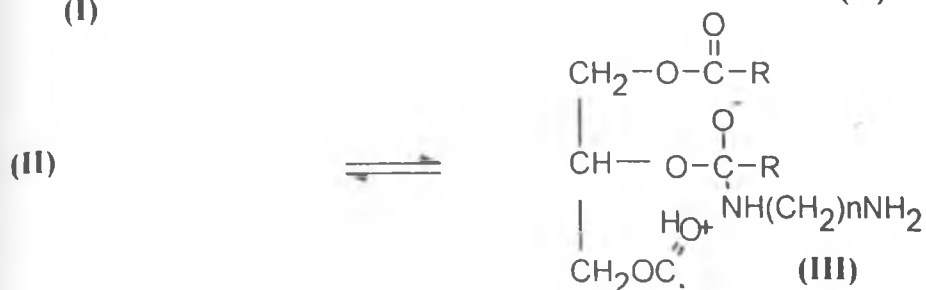
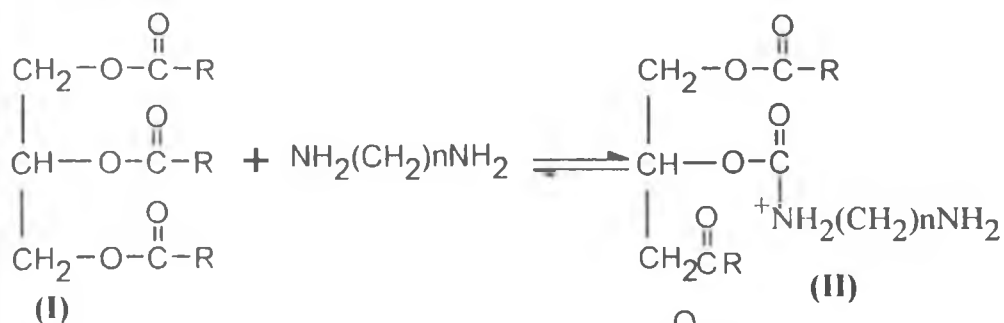
Vernonia oil was found to contain other fatty acids such as oleic, linoleic, stearic, palmitic, etc., thus making it difficult to obtain pure vernolic acid derivatives. On treating the vernonia oil with aliphatic diamines, aminolysis took place at ester group giving rise to the corresponding fatty acid bisamides and aminoamides depending on the condition set. The yields of both amides depend on temperature, molar ratio and the concentration of the reactants. Highest yields of aminoamide were obtained at room temperature using molar ratio of 1:5 (vernonia oil : diamine) without any solvent. Highest yields of diamide were obtained using molar ratio of 1:3 (vernonia oil : diamine) at refluxing temperature (61°C) of chloroform. Due to multiple functional groups in the vernolic acid such as the ester, double bond and epoxy ring, higher temperatures above 70°C would lead to the opening of epoxy ring giving rubber-like product. Unlike aliphatic diamines, aromatic diamines do not react with the ester group of vernonia oil at low temperatures but react with the epoxy group at higher temperatures or in the presence of catalyst giving rise to polymeric products.

The amidation of the oil was found to be faster than that of methyl esters. The completion of the triglyceride reaction with diamines ranged between 5-15 hours whereas the reactions involving methyl vernolate did not go to completion within fifteen hours. The fast rate of amidation of the triglyceride can be attributed to the removal of a proton from the amino group (that has already become attached to the carbonyl of the first ester group) by the carbonyl of the other ester groups within the triglyceride molecule. This intramolecular proton transfer leads to activation of the ester groups towards being attacked by the second amino group. In the case of aminolysis of methyl esters, a second molecule of diamine is required to pull out the proton at the transition state.

The following equation represents a possible mechanism of the amidation of methyl esters



The following equation represents a possible mechanism of the amidation of triglyceride



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APPENDIX

Spectra GC, HPLC, IR, NMR and MS

FIGURE. 4

GC / MASS SPECTRUM OF METHYL ESTERS OF VERNONIA OIL.

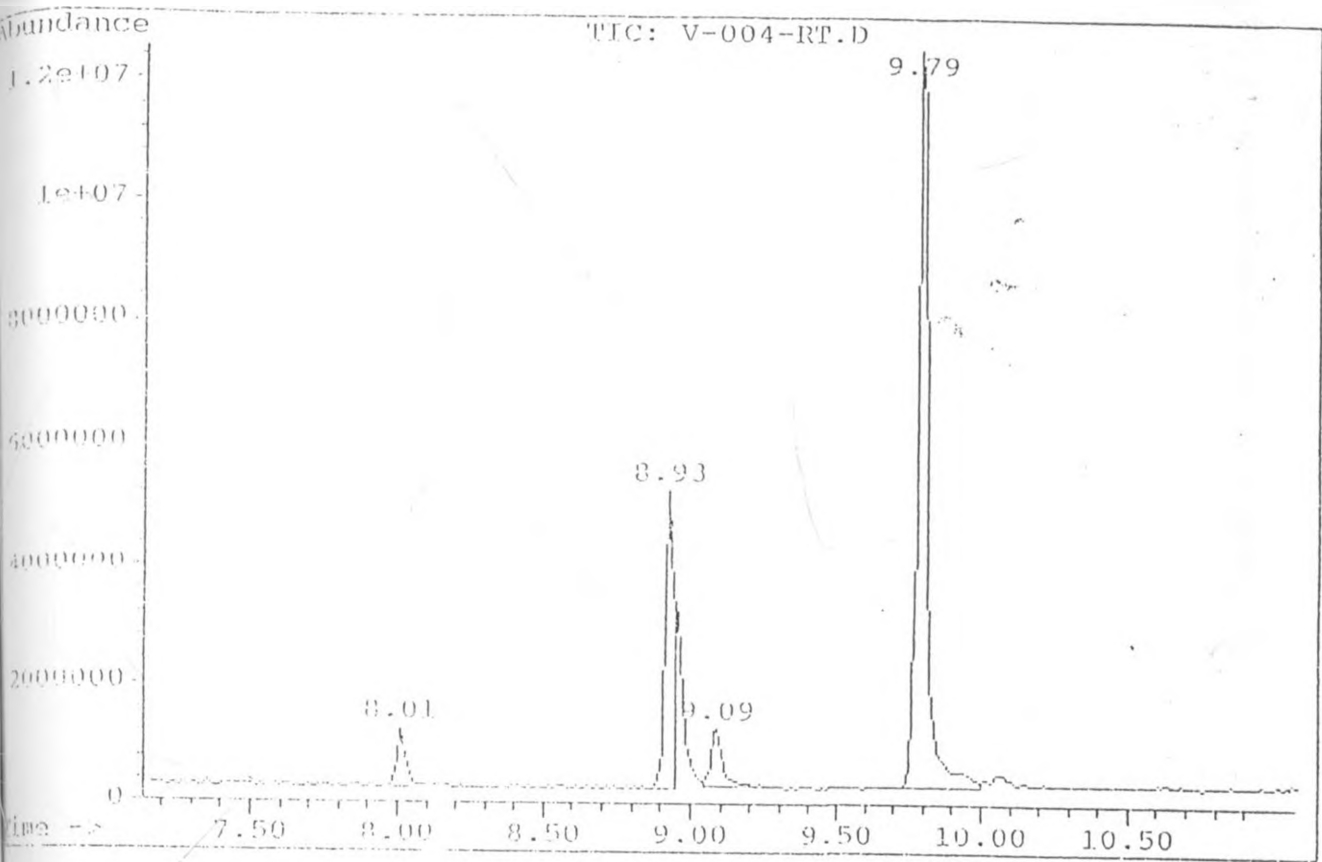


FIGURE 5 IR SPECTRA OF METHYL VERNOLATE

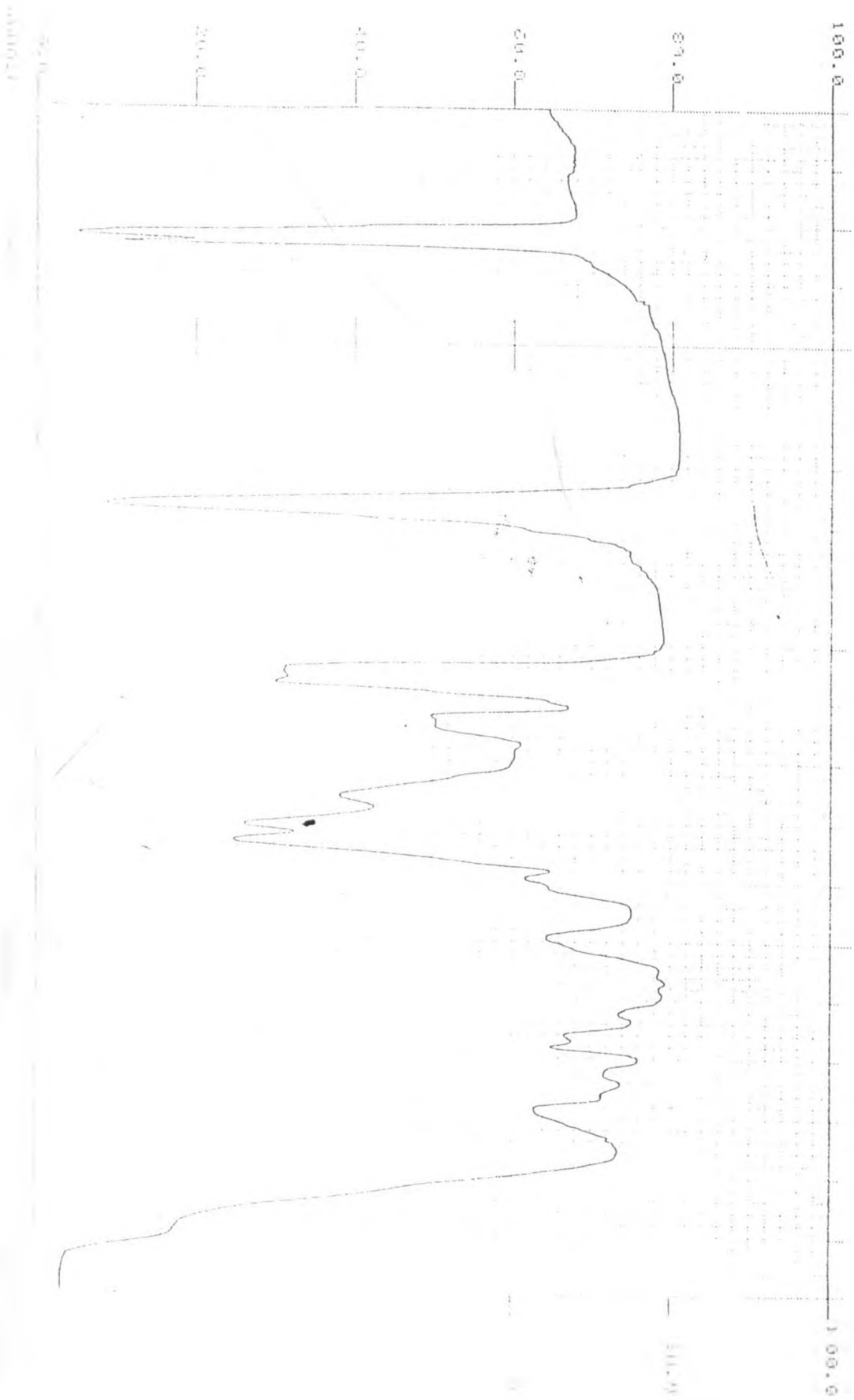


FIGURE 6. GC/ MASS SPECTRUM OF METHYL VERNOLATE.

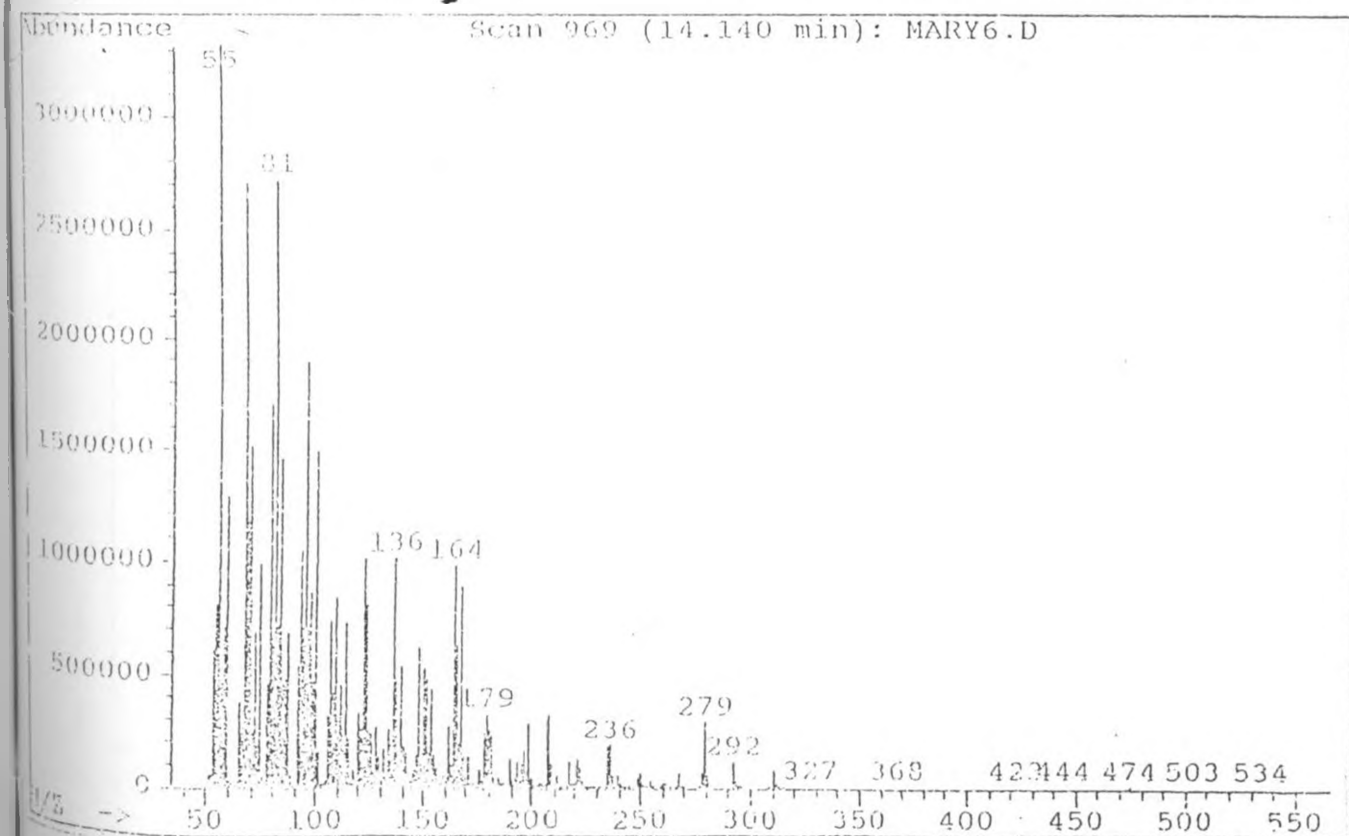
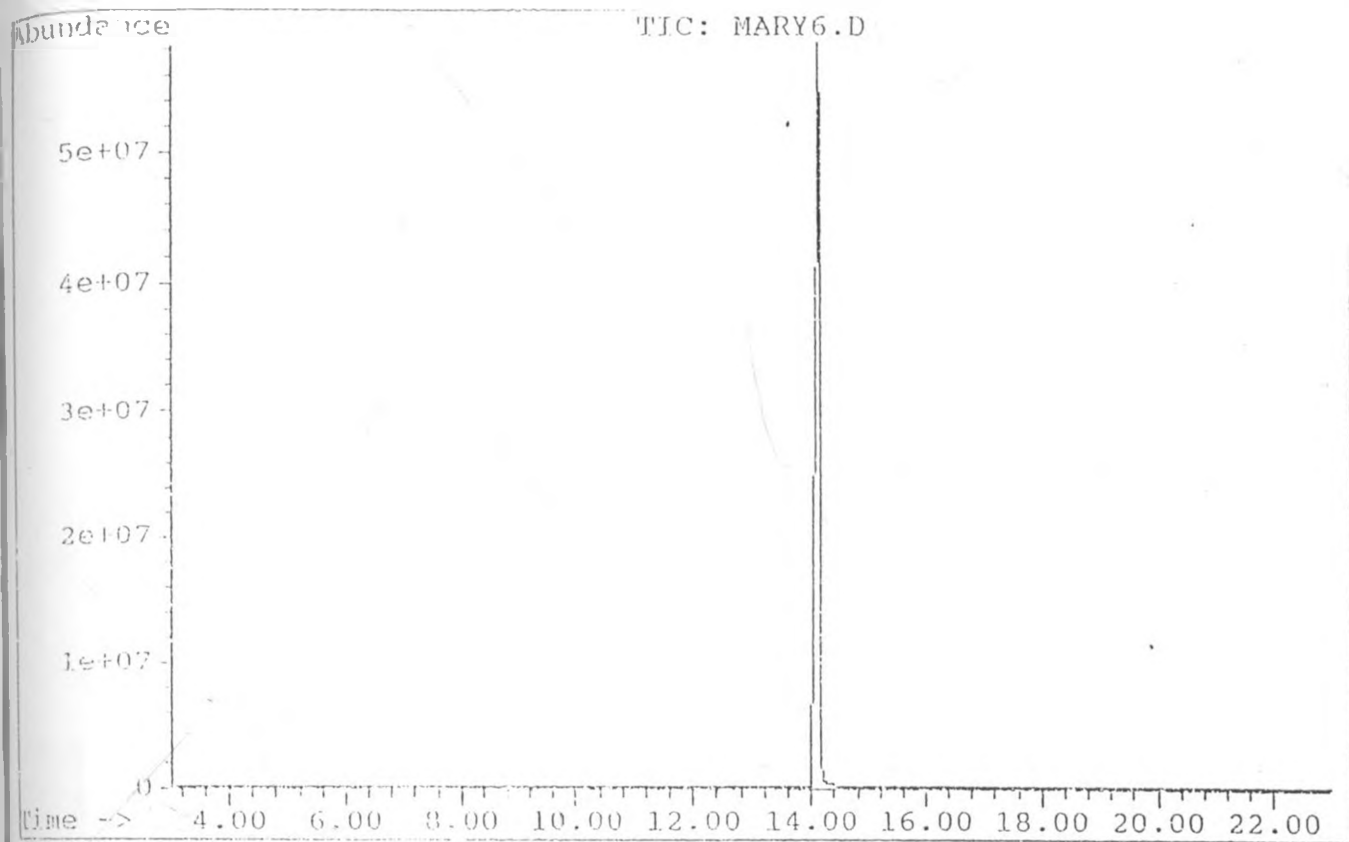


FIGURE 7. IR SPECTRA OF VERNONIA OIL

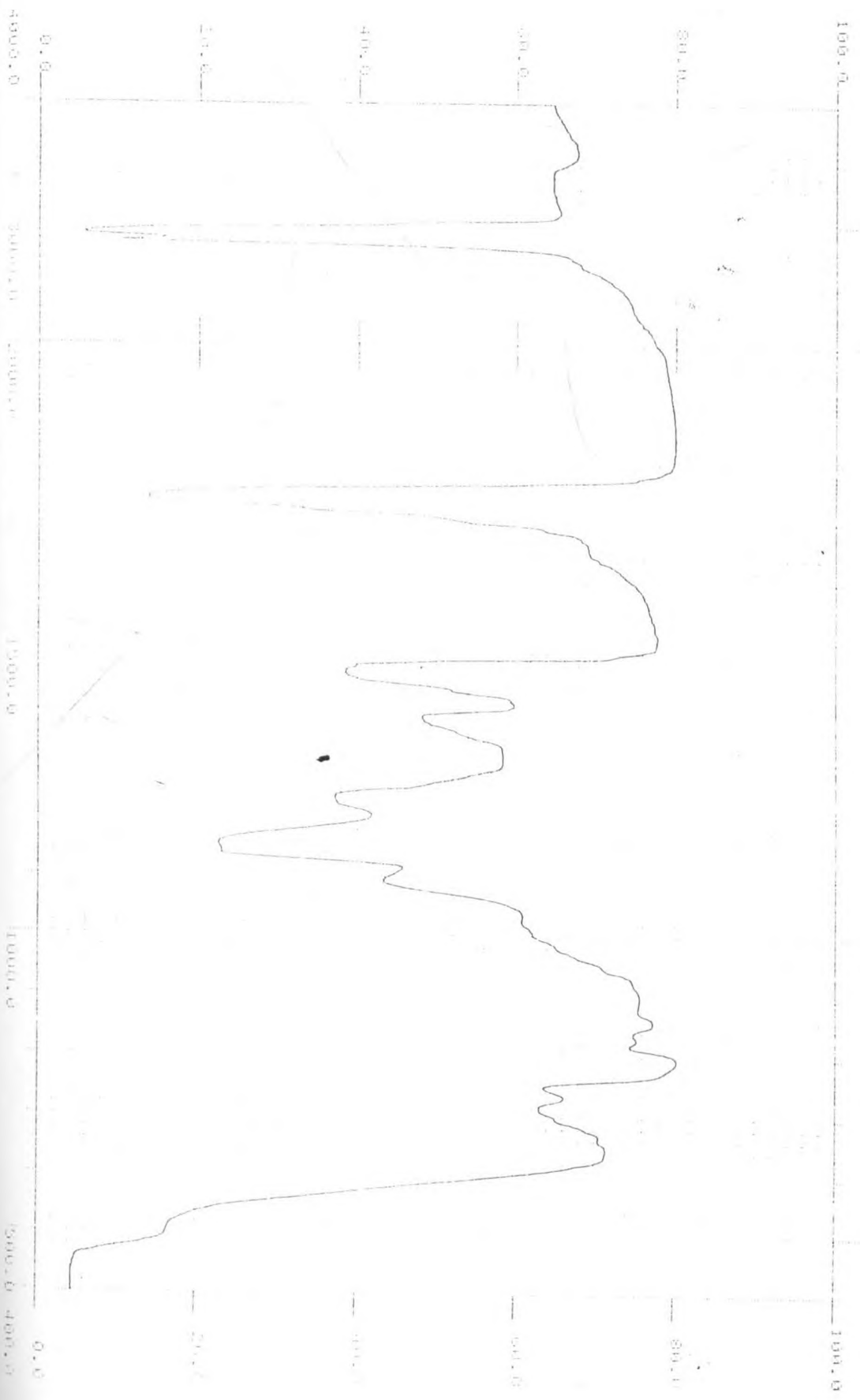
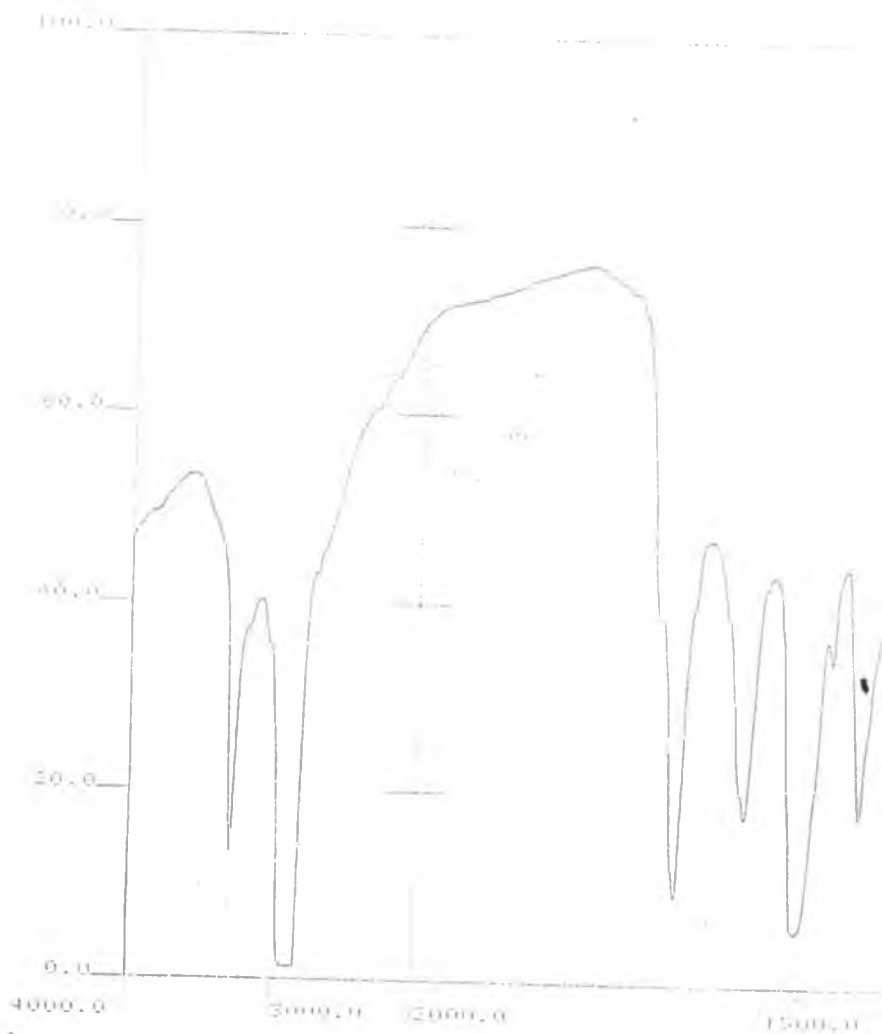
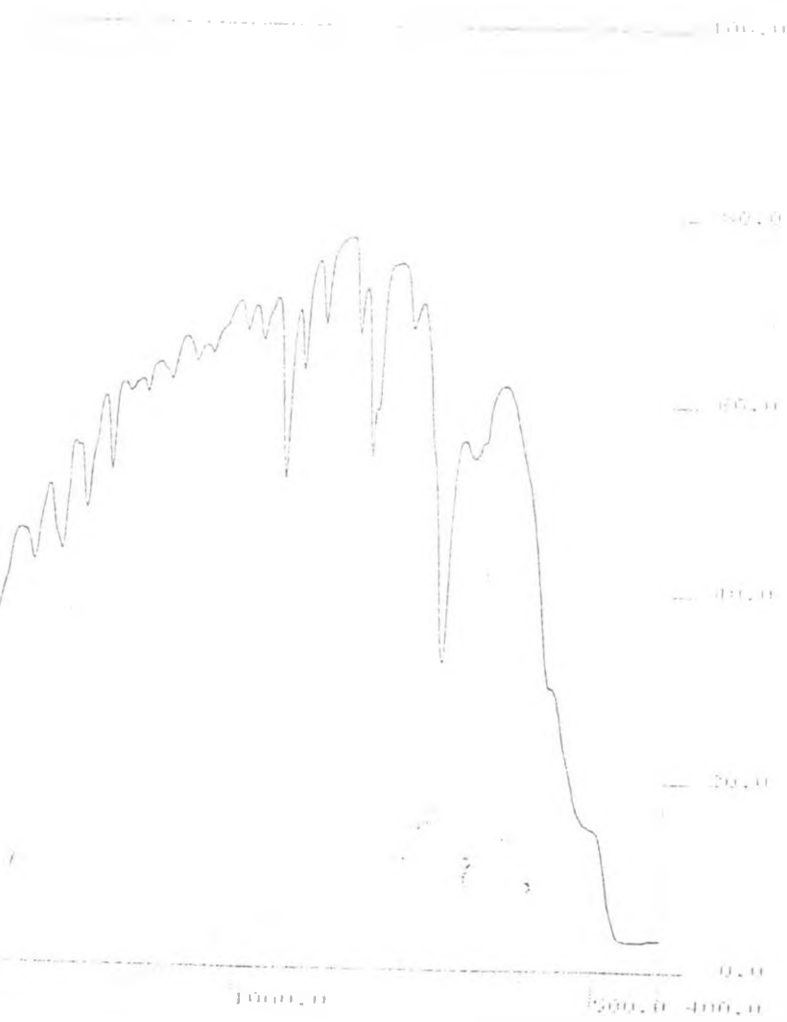


FIGURE 8. IR SPECTRA OF N-(4-AMINOBTUTYL)VERNOLAMIDE.





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FIGURE 9. ¹H-NMR SPECTRA OF N-(4-AMINOBTYL)VERNOLAMIDE.

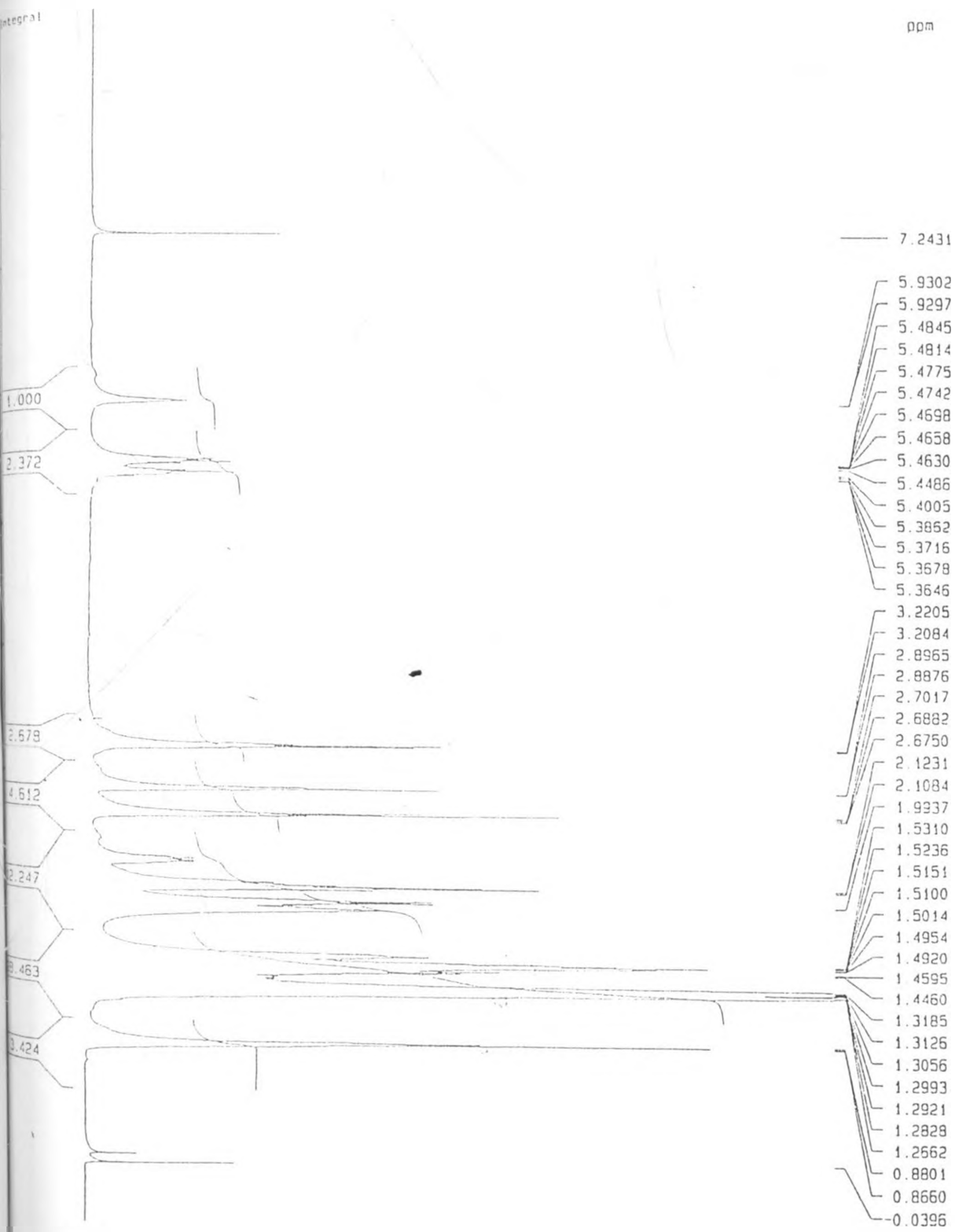


FIGURE 10 ¹³C-NMR SPECTRA OF N-(4-AMINOBTYL) VERNOLAMIDE

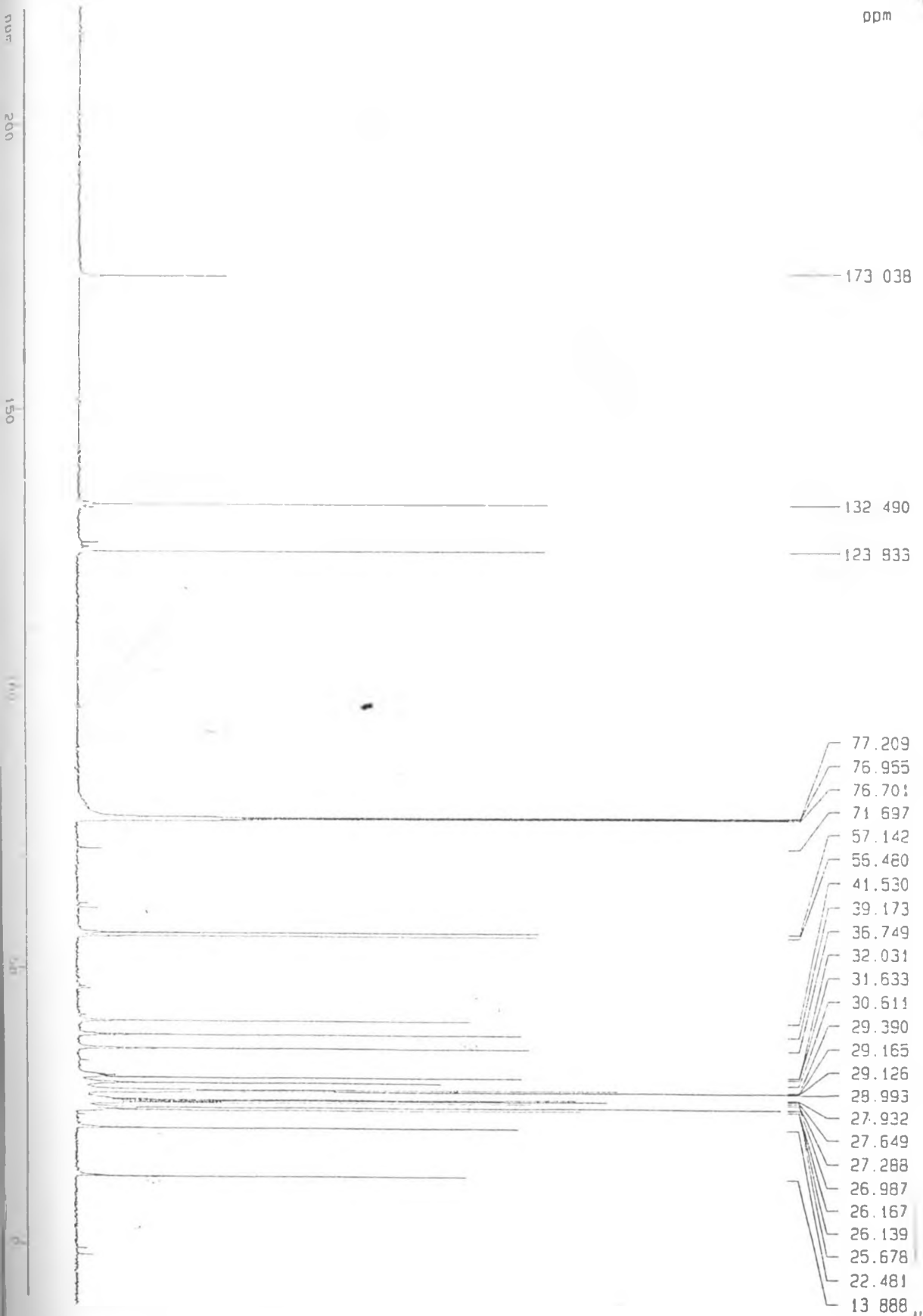


FIGURE 11 IR SPECTRA OF N,N'-TETRAMETHYLENE BIS(VERNOLAMIDE)

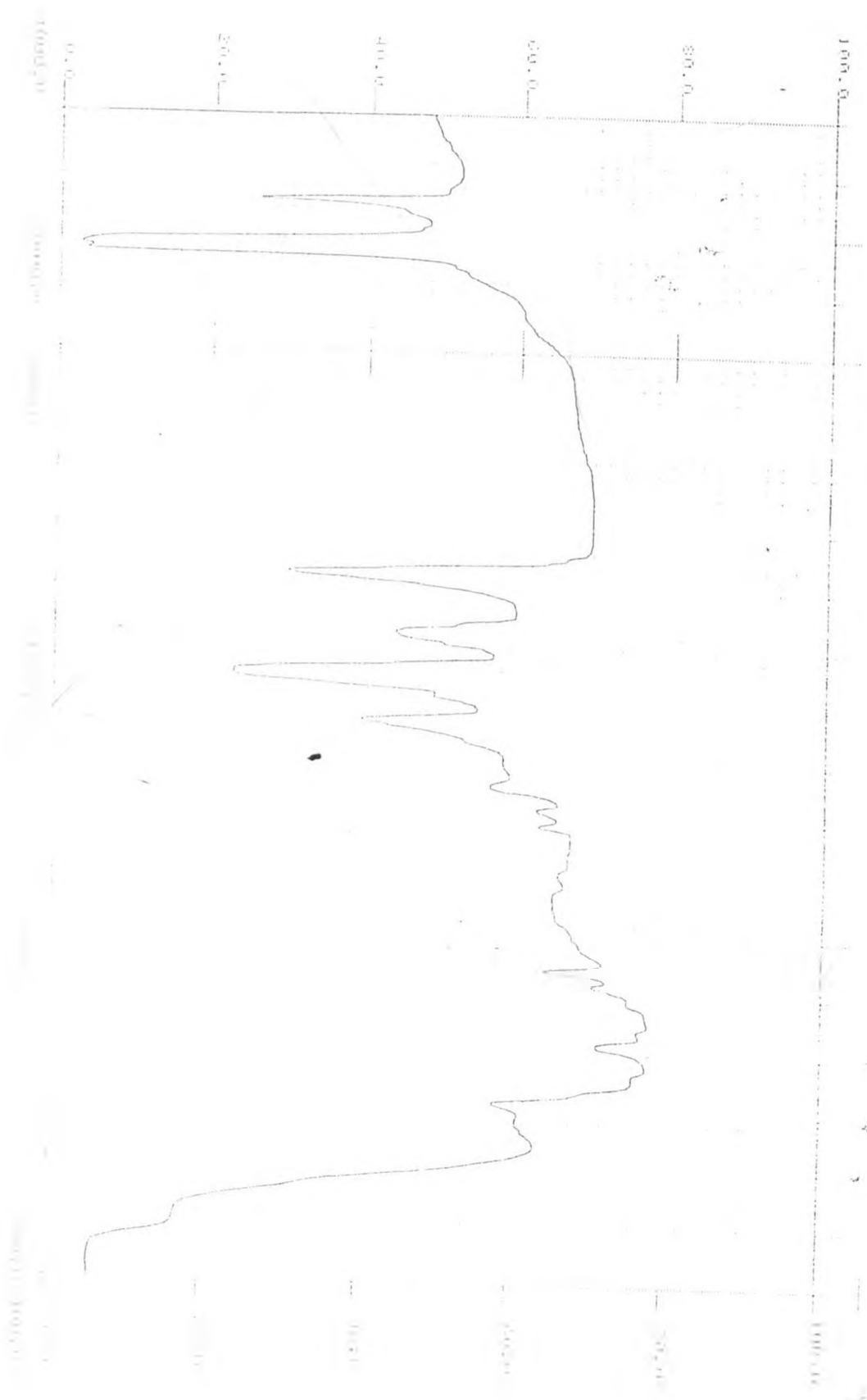


FIGURE 12 ¹H-NMR SPECTRA OF N,N'-TETRAMETHYLENE BIS(VERNOLAMIDE)

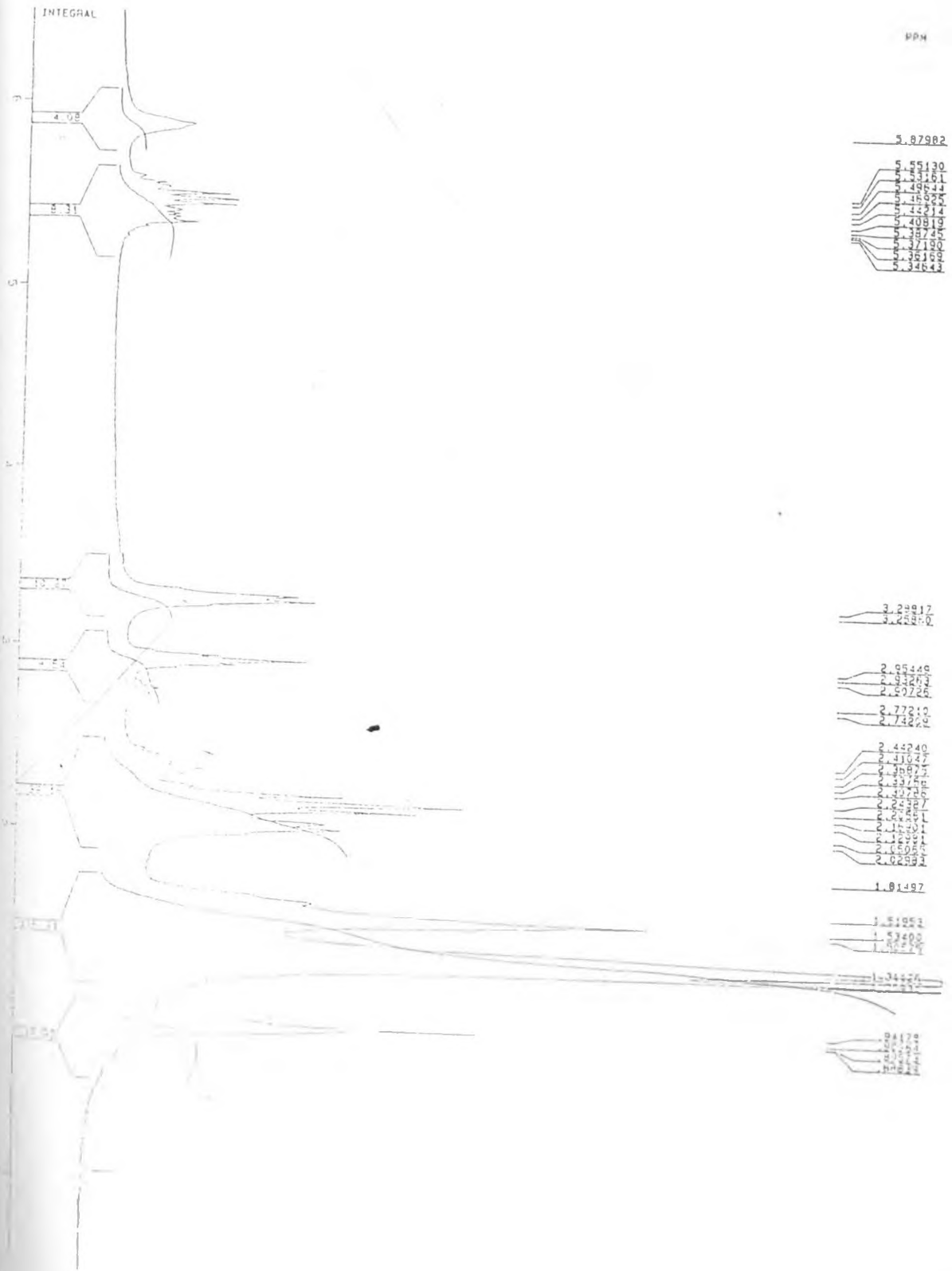


FIGURE 13. ¹³C-NMR SPECTRA OF N,N'-TETRAMETHYLENEBIS(VERNOLAMIDE)

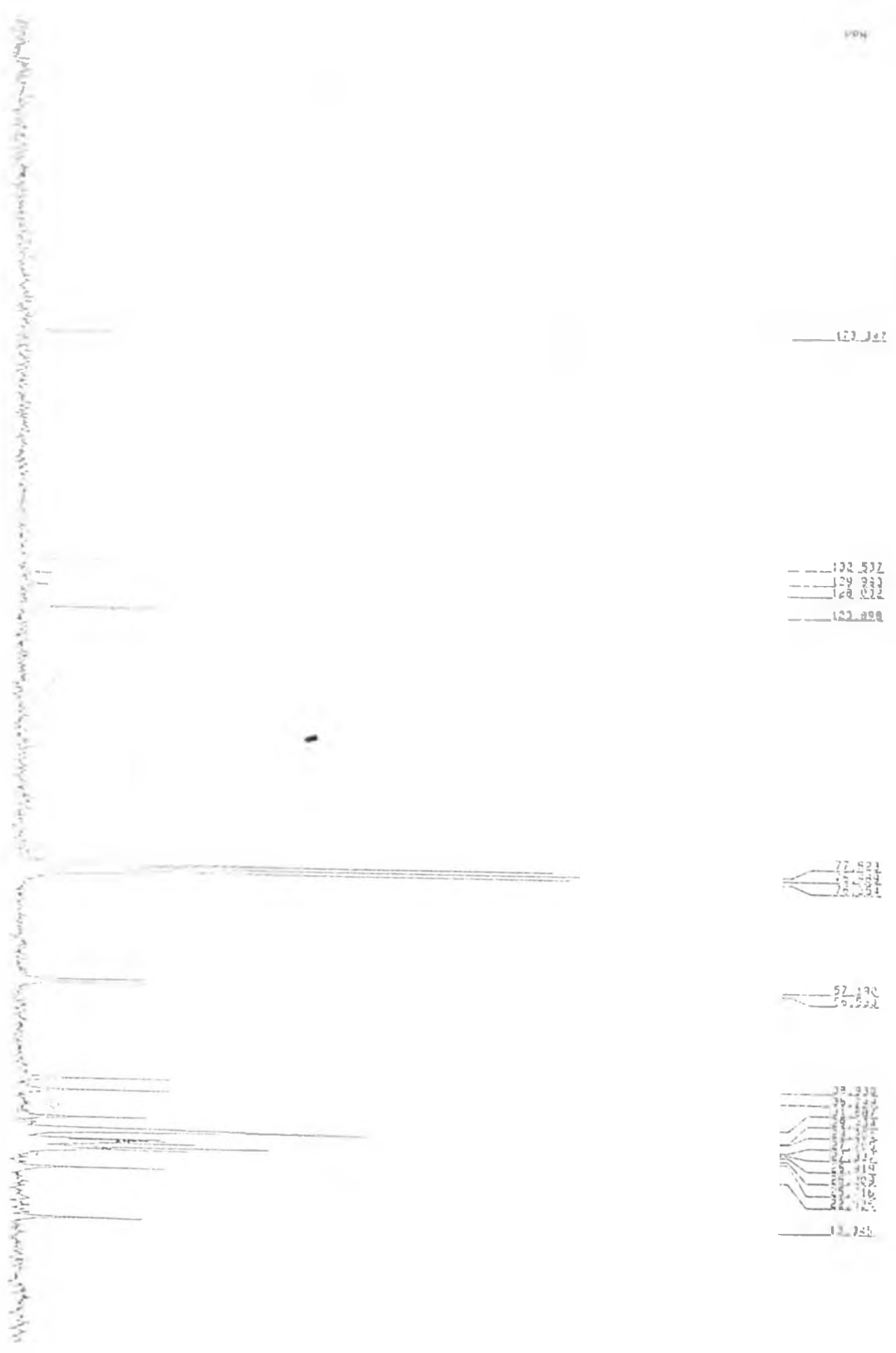
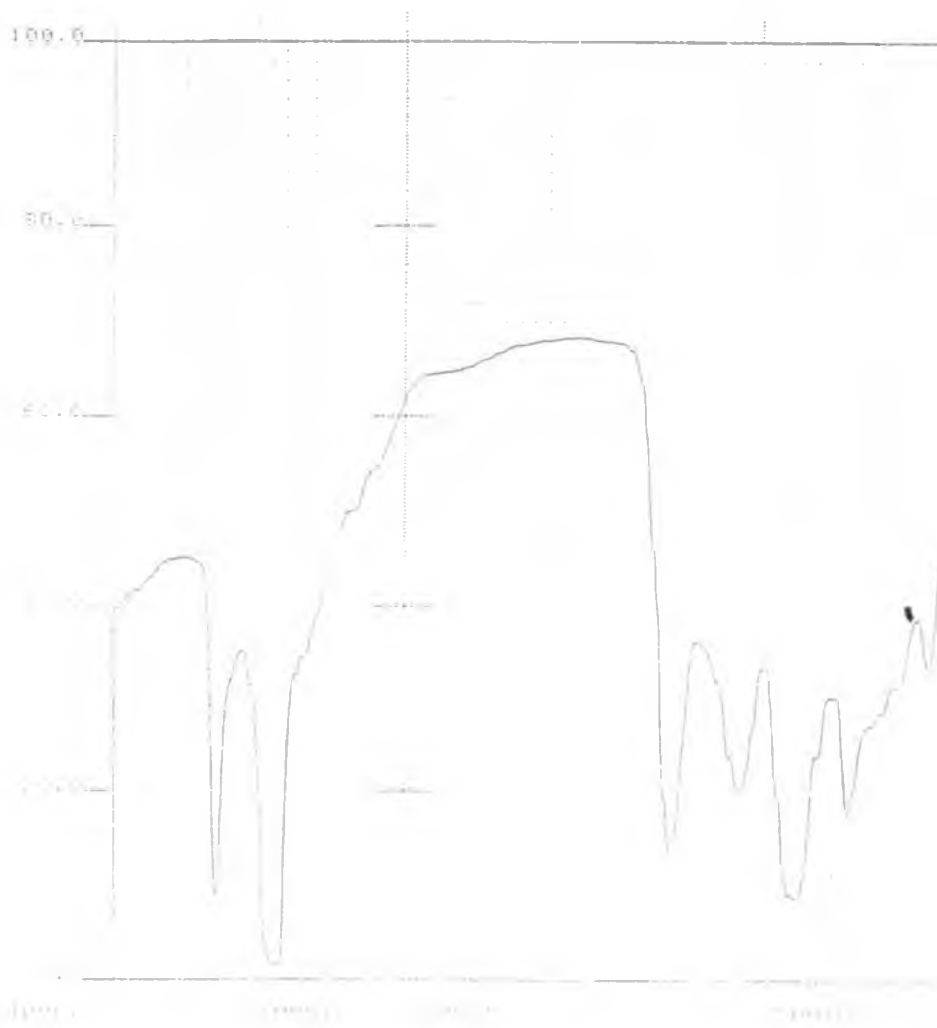


FIGURE. 14. IR SPECTRA OF N-(4-AMINOBTYL)VERNOLAMIDE.



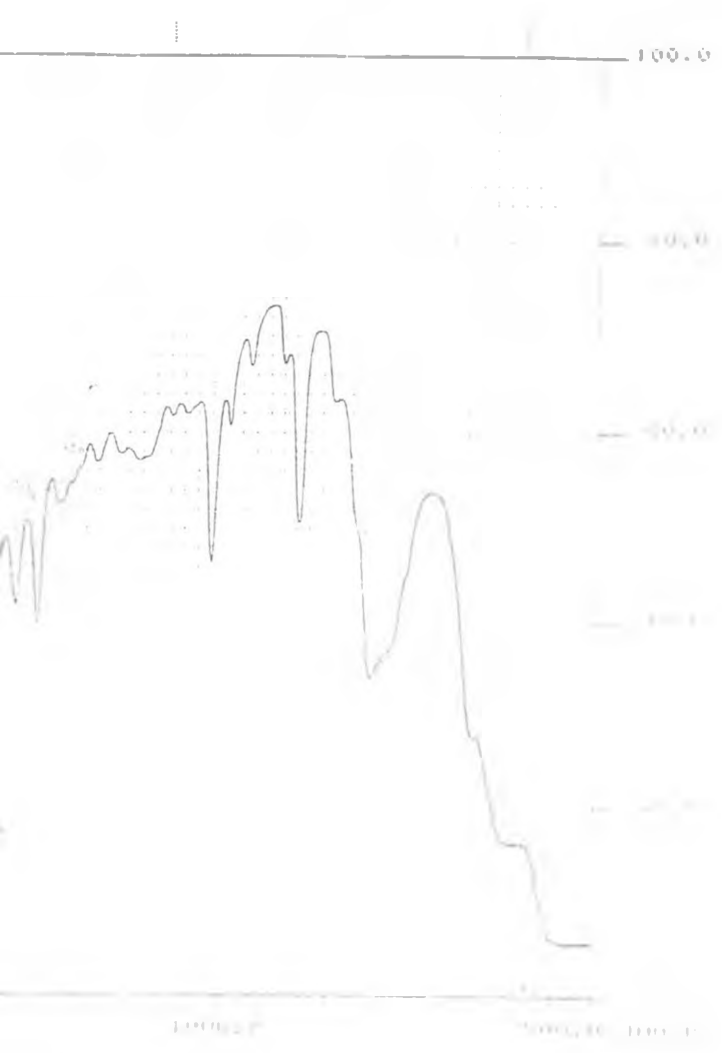


FIGURE 15. ¹H-NMR SPECTRA OF N-(4-AMINOBTYL)VERNOLAMIDE.

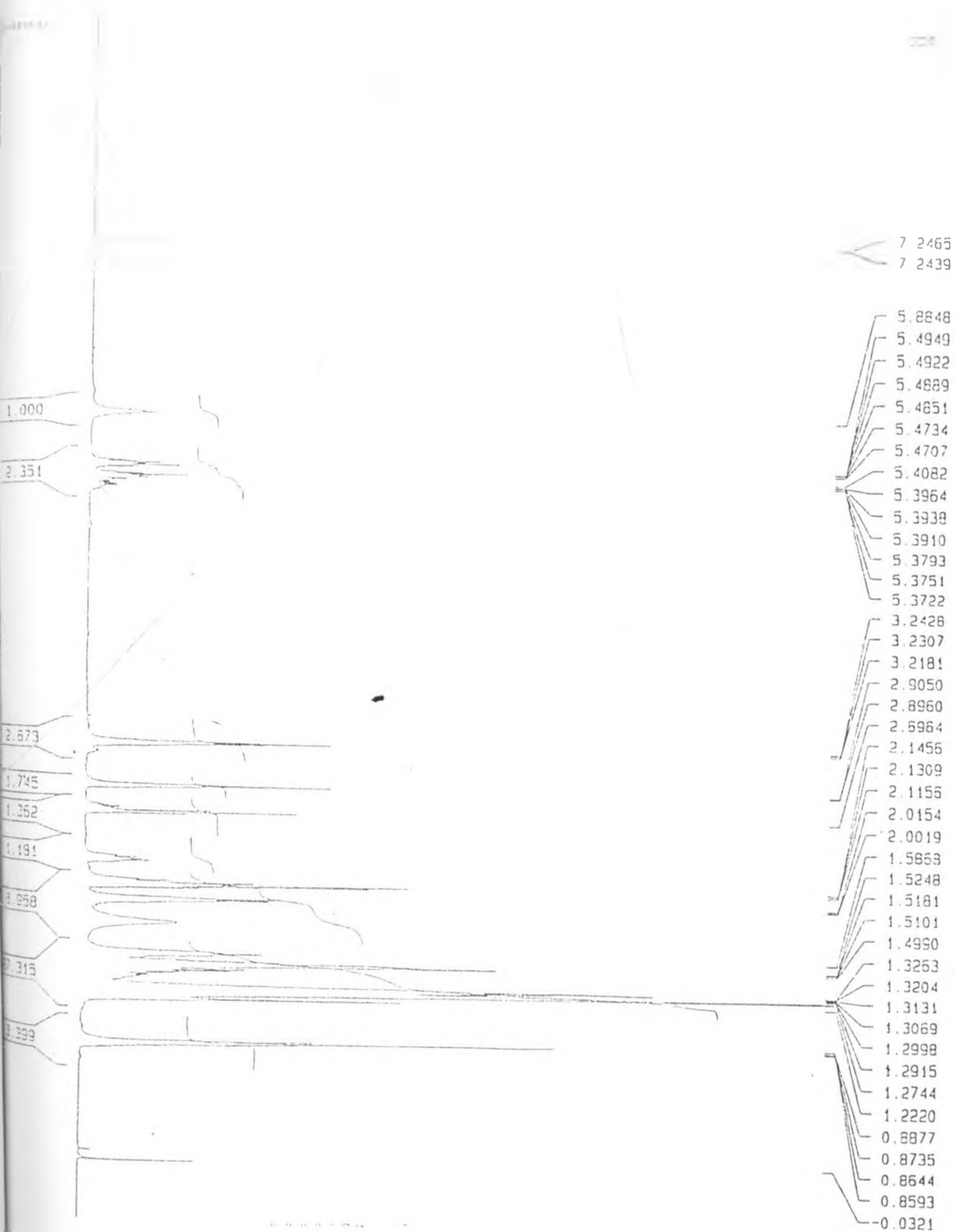


FIGURE 16.

¹³C-NMR SPECTRA OF N-(4-AMINOBTYL)VERNOLAMIDE.

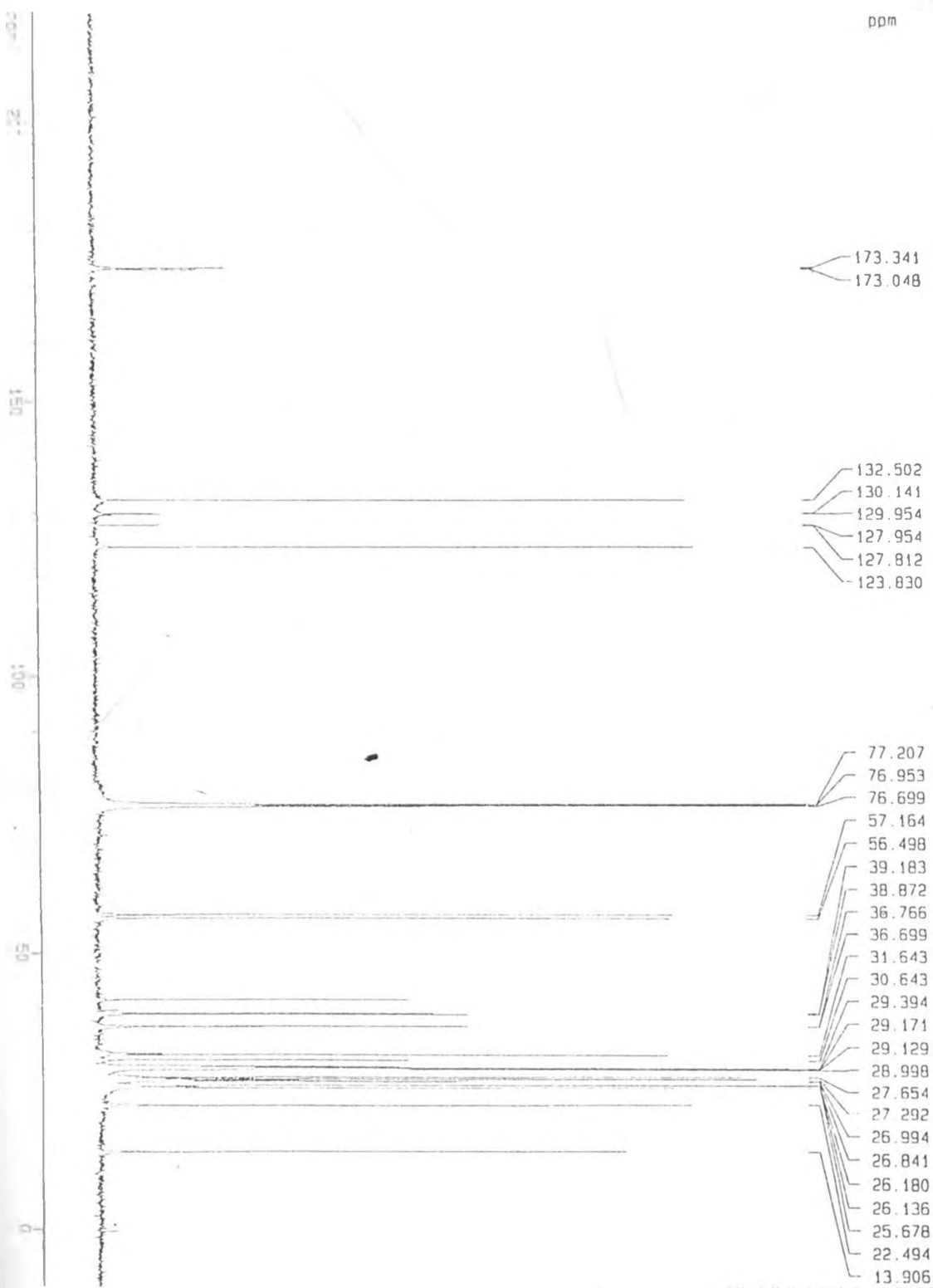


FIGURE 17. IR SPECTRA OF N,N'-TETRAMETHYLENE BIS(VERNOLAMIDE)

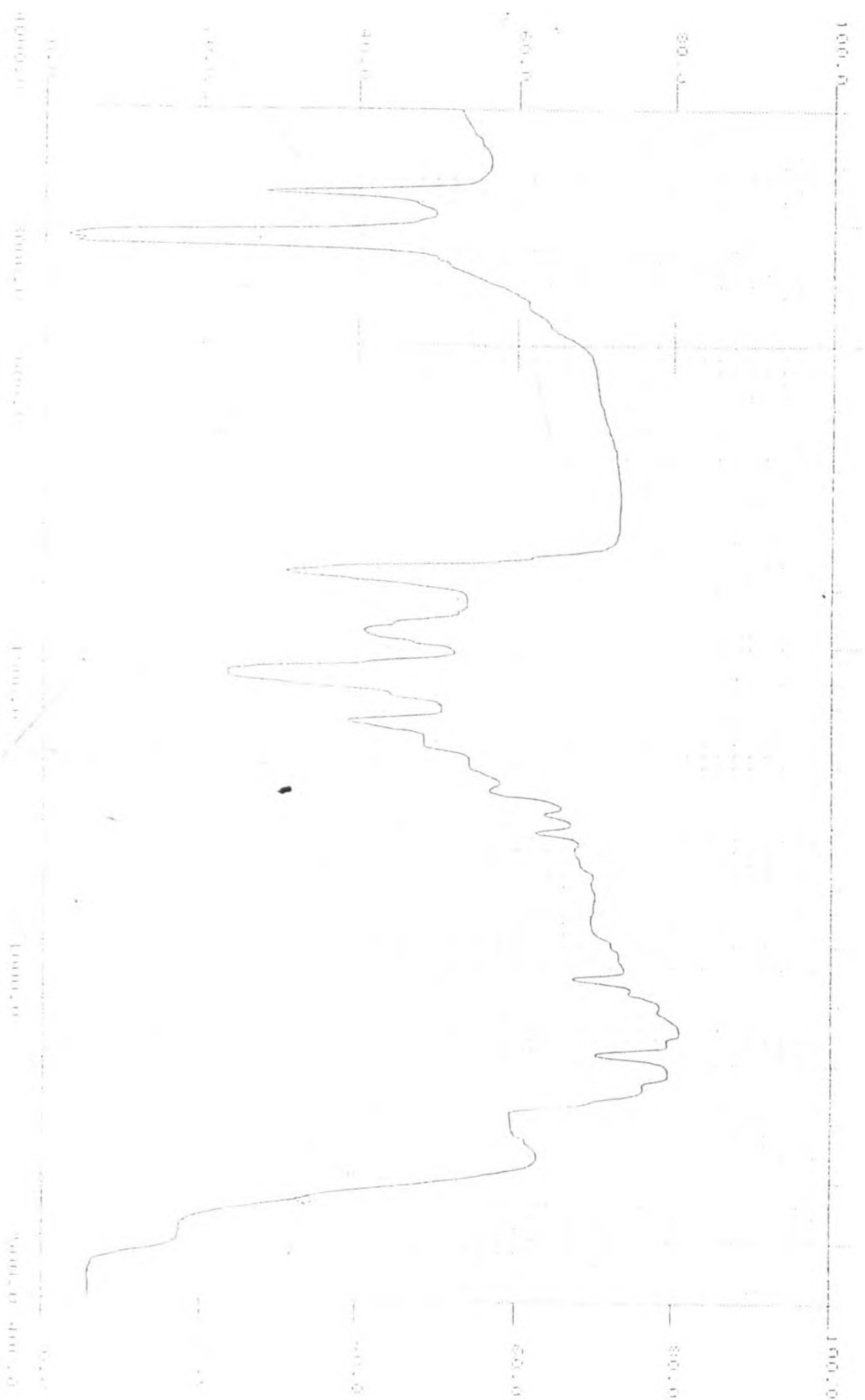


FIGURE 18.

¹H-NMR SPECTRA OF N,N'-TETRAMETHYLENE BIS(VERNOLAMIDE).

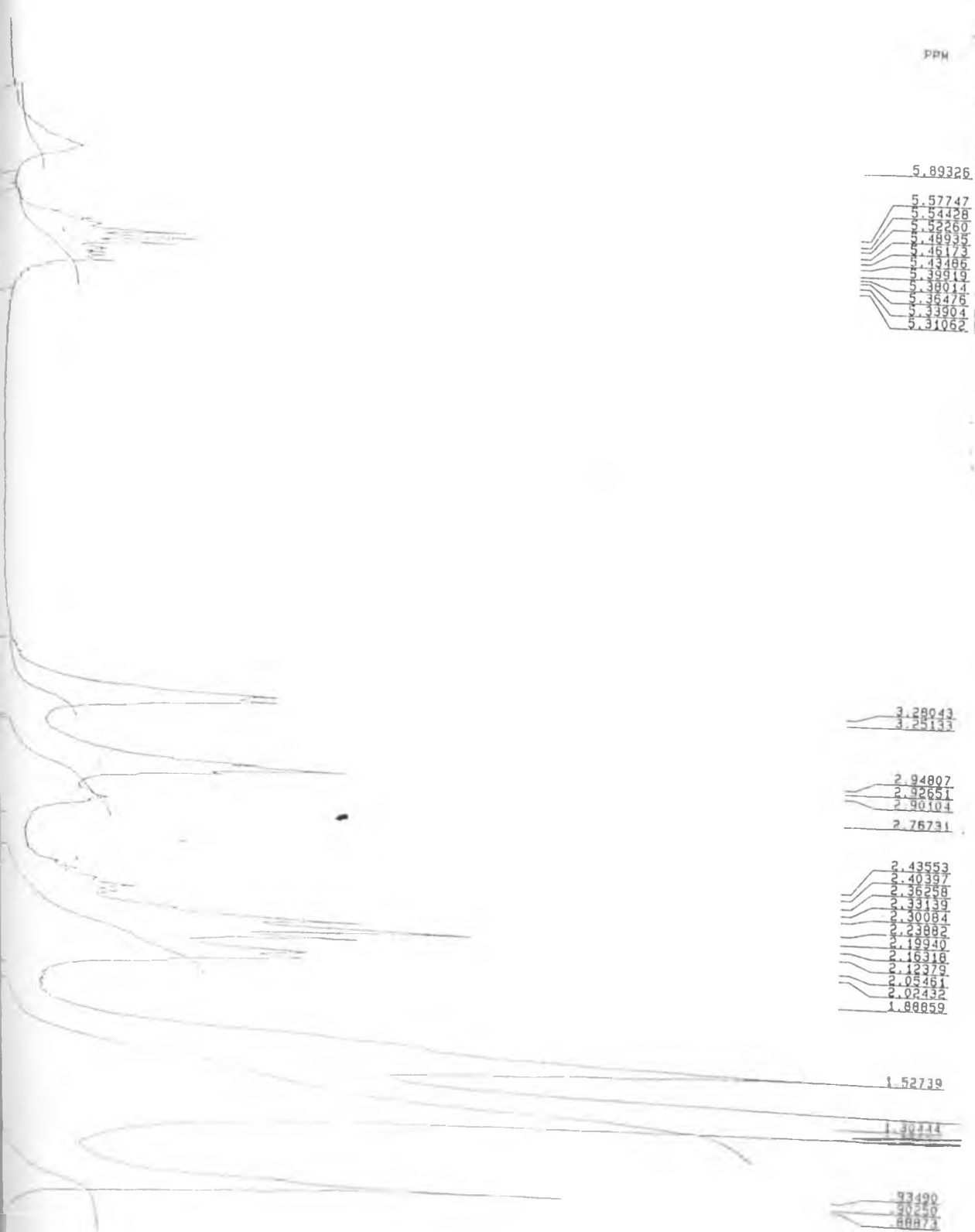


FIGURE 19. ¹³C-NMR SPECTRA OF N,N'-TETRAMETHYLENE BIS(VERNOLAMIDE)-

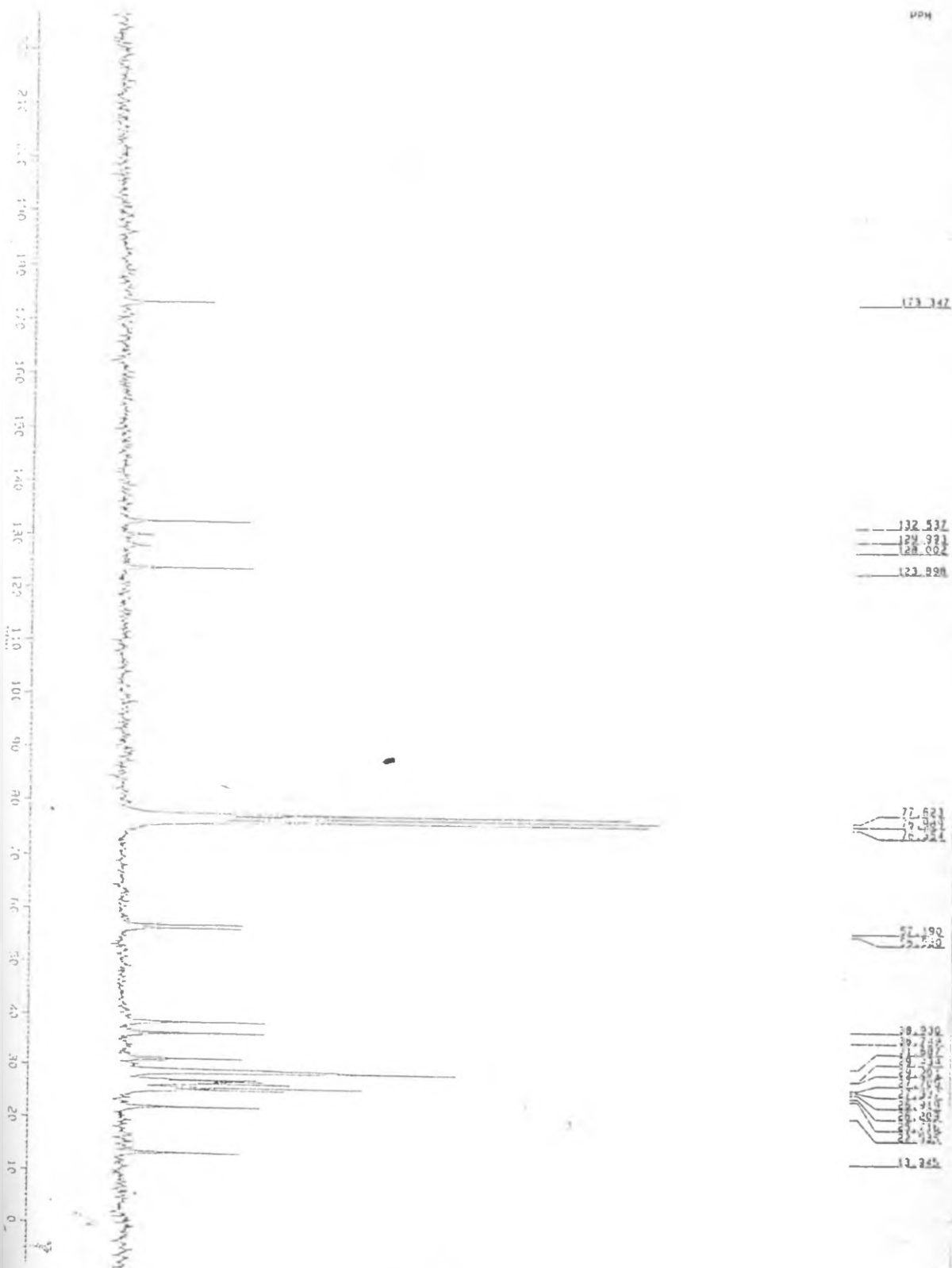


FIGURE 20 IR SPECTRA OF 1,6 DIAMINOHEXANE PRODUCT

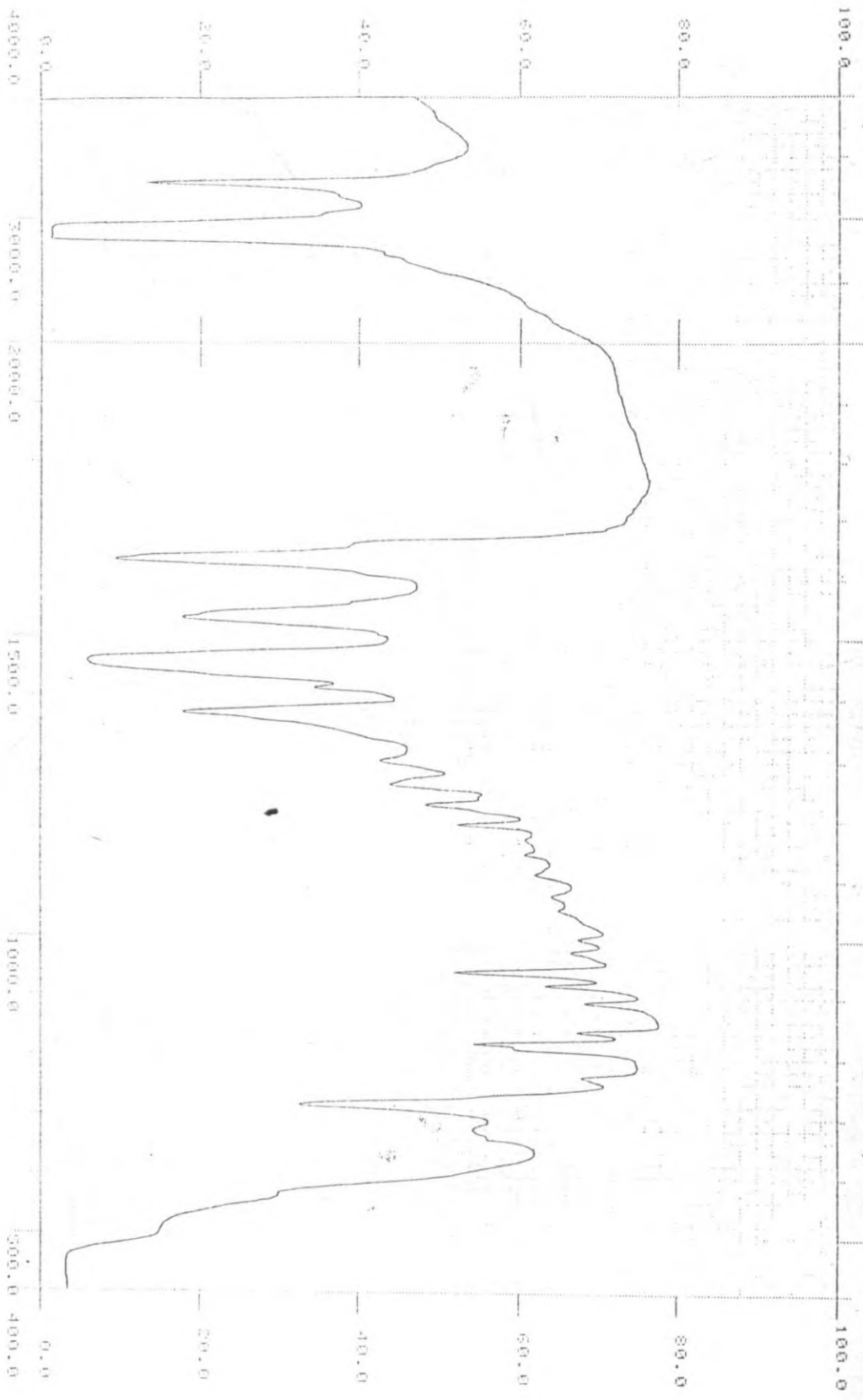
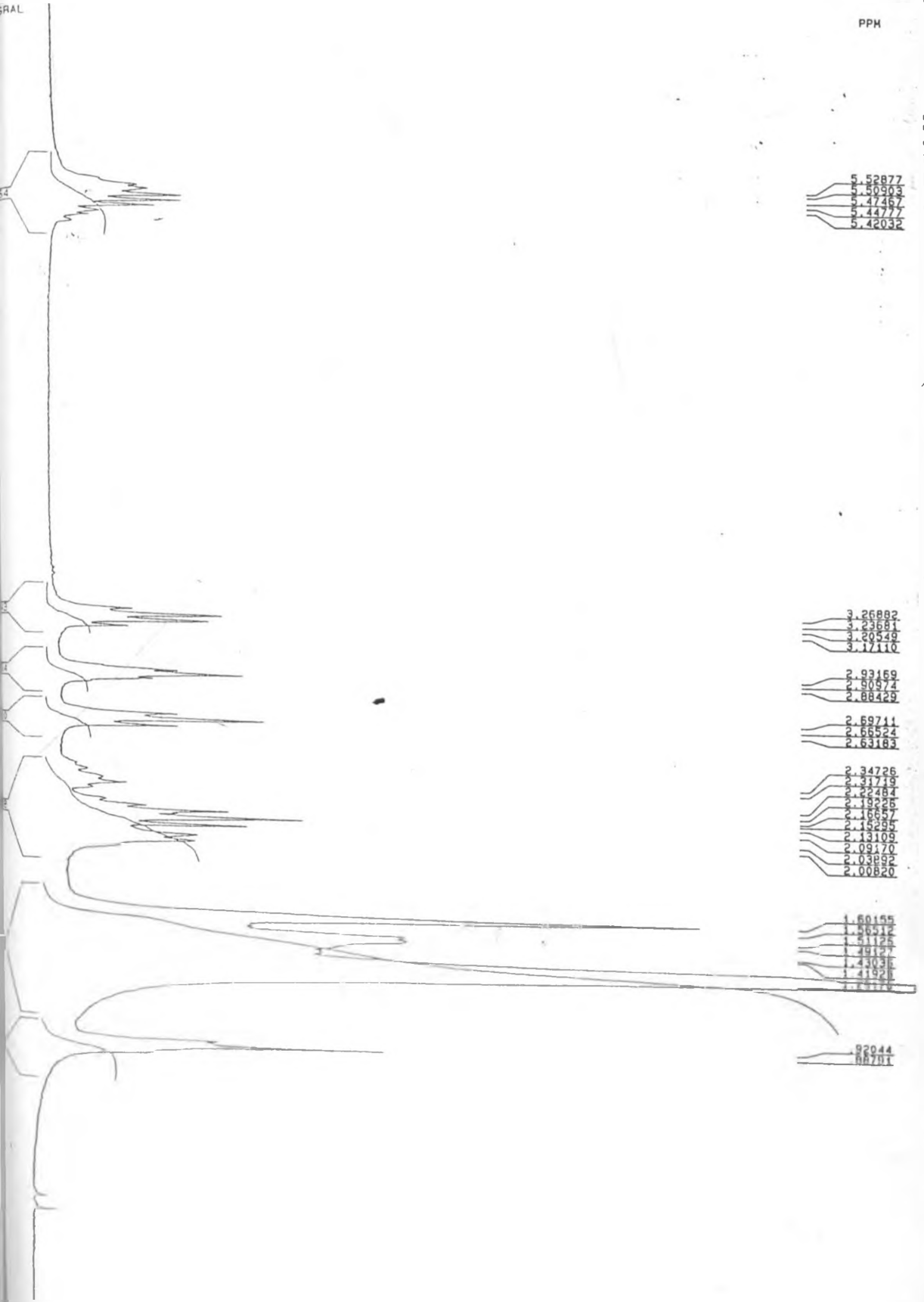
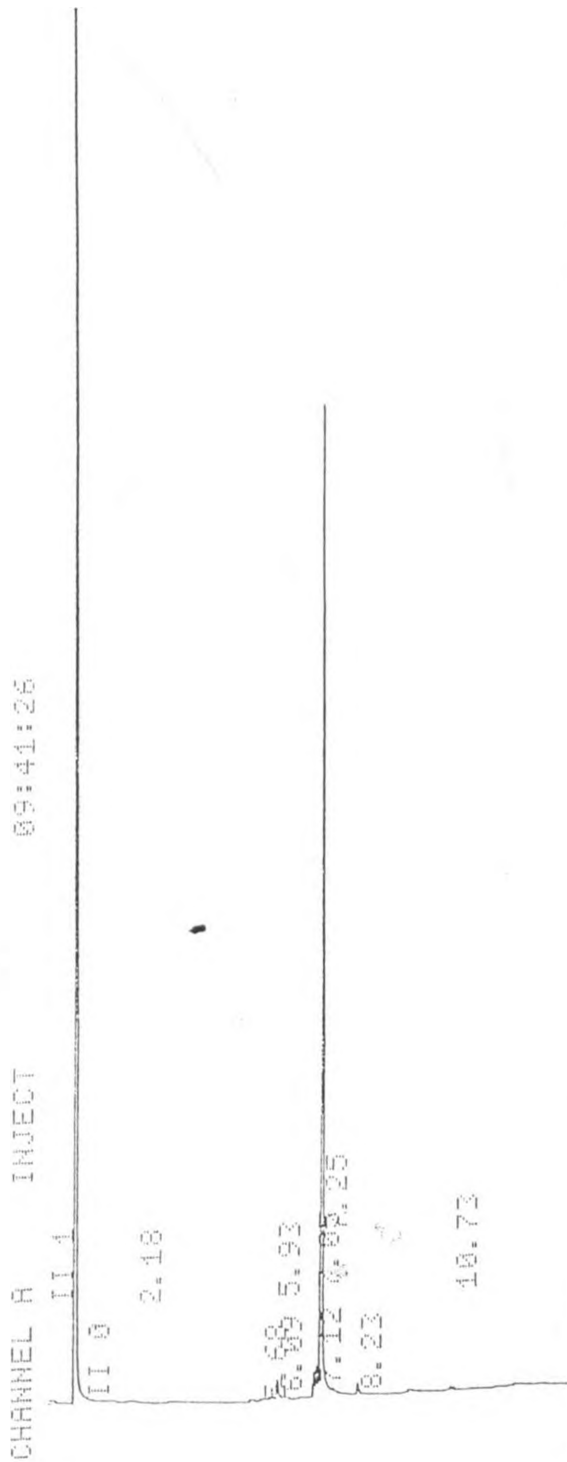


FIGURE 21.

¹H-NMR SPECTRA OF N-(6-AMINOHEXYL)VERNOLAMIDE





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FIGURE. 24 HPLC SPECTRA OF N,N'-HEXAMETHYLENE BIS(VERNOLAMIDE)

