THE SYNTHESIS OF VERNOLAMIDES FROM *VERNONIA GALAMENSIS* OIL

BY:

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DECLARATION

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

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ABSTRACT

Vernonia oil extracted from *Vernonia galamensis* seeds constitutes a viable synthetic starting material for obtaining new chemical derivatives with higher added value. The vernonia oil was obtained by soxhlet and cold extraction of *V. galamensis*. The percentage yield of crude oil extracted ranged from 19-31%. Losses of up to 5% of the oil were incurred during the refining process, mainly due to the charcoal treatment (5%). The reaction of vernonia oil with some primary amines resulted in the formation of epoxy amides. The reactions proceed even at room temperature, but for optimum yields, it was done at 50°C.

Vernonia oil was reacted with 1-(2-aminoethyl)pyrrolidine, 1-(3-aminopropyl)imidazole, 4-(3-aminopropyl)morpholine and 1-(3-aminopropyl)-2-pyrrolidinone (molar ratio 1:3) at 50°C to obtain the respective vernolamides: N-2-(1-pyrrolidino)ethylvernolamide, N-3-(1-imidazole)propylvernolamide, N-3-(4-morpholino)propylvernolamide and N-3-[1-(2-oxopyrrolidino)]propylvernolamide. The reactions were complete after 16 -18 hours except for the reaction of vernonia oil with 1-(3-aminopropyl)imidazole which was done for 48 hours in dimethylformamide.

On further reaction of N-2-(1-pyrrolidino)ethylvernolamide with dilute acid and acetic acid at room temperature, the epoxy group was opened up to give 12, 13-dihydroxy-N-2-(1-pyrrolidino)-ethyl-9-octadecenamide and hydroxy ester derivatives. This demonstrates the potential for the development of new applications.

The derivatized compounds were analysed by thin-layer chromatography (TLC), infrared (IR), elctron impact mass spectroscopy (EI MS), chemical ionization (CI) and nuclear magnetic resonance (NMR) spectroscopic techniques.

The antimicrobial activities of the vernolamides were investigated. The vernolamides exhibited both antibacterial and antifungal activity. Both the antibacterial and antifungal tests were investigated at concentrations of 100 μg, 50 μg and 25 μg by the disc method. For the antifungal tests, the vernolamides showed inhibition against *Saccharomyces cerevisiae, Trichophyton mentagrophyte* and *Microsporum gypseum*. Moreover, the activity of the compounds was more pronounced against the gram-negative bacteria (*Escherichia coli*) where 20 - 30 mm in diameter (inhibition zones) were recorded as compared to the activity against *Staphylococcus aureus* (gram-positive) and *Bacillus subtilis*.

CHAPTER ONE

INTRODUCTION

1.0 General

The discovery of vernolic acid (*cis*-12,13-epoxy-*cis*-9-octadecenoic acid) (Fig.1A) in *Vernonia anthelmintica* by Gunstone (1954) intensified the research for new sources of industrial raw materials. The focus was on new and unique plant constituents that would not compete with those then in adequate supply and that could be used to satisfy existing needs or anticipated needs. This included the discovery of unusual seed oils for which new industrial markets might be created or might recapture markets lost by agricultural products to petrochemicals.

Seeds from vernonia plants were screened for oil content and those with substantial amounts were evaluated to identify oils with unique fatty-acid composition, distinctly different from oils of peanut, cottonseed, soybean, linseed or other domestic crops [Perdue et al., 1986]. Substantial quantities of epoxy oils were then used by industry to manufacture plastic formulations, protective coatings and other products. Existing needs were met with petrochemicals or by chemical modification (epoxidation) of fats and vegetable oils such as soybean and linseed oils; hence, the prospects of naturally occurring epoxy acids into the markets was good. Epoxidizing these inexpensive and readily available unsaturated vegetable oils increases their value two to threefold. It is the epoxy groups of such triglyceride oils that make these materials useful in plastics and coating products. They serve as plasticizers, stabilizers and generally as highly reactive sites where one triglyceride molecule can become attached to adjacent molecules to form interlocking polymer networks.

Among epoxy-containing seed oils, the most promising species appear to be *Vernonia anthelmintica*, *Stokesia Laevis*, *Euphorbia lagascae* and *Vernonia galamensis*. Many factors that

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influence selection of species for commercial epoxy oil production include percentage of epoxy acid in the oil; oil content of the seed; seed yield per acre; agronomic characteristics of the species; non-epoxy constituents in the oil; physical properties of the oil and composition of the seed meal.

While most triglyceride oils contain only double bond functionality, castor oil and vernonia oils in addition to the double bond contain hydroxyl and epoxy groups, respectively. *Vernonia galamensis* is a new industrial oil seed crop. It is a good source of a reactive diluent that could substantially reduce air pollution, at least in oil-based paints *Vernonia galamensis* produces high quantities of epoxy fatty acids useful in the reformulation of oil-based (alkyd-resin) paints to reduce emission of volatile organic compounds that contribute to production of smog [Perdue et al., 1986]. Other potential markets for the fatty acids include polymer blends and coatings, cosmetic and pharmaceutical applications . About 38% of the seed is the oil which contains about 72% vernolic acid. The unique structure of vernolic acid, makes it have a much wider use than epoxidized oils. Further epoxidation of this oil would require only about half the cost of soybean and linseed oils [Carlson and Chang, 1985].

The transformation of vernonia oil into key industrial raw materials has been a major focus recently [Ayorinde et al., 1988, 1989; Grinberg et al., 1994] ,especially its transformation into dibasic acids, hydroxy alkoxy fatty acids and fatty amides. Fatty amides are important chemical intermediates for commerce, with applications ranging from paper coatings and printing ink additives to slip and anti-block additives for polyethylene films [Bryant et al., 1993].

Epoxides play a very important role in biological processes. The reactivity of epoxides toward nucleophilic ring opening is responsible for one of the biological roles they play. Mhaskar and co-workers (1993) synthesized *N*-acyl-L-leucines of some uncommon fatty acids and correlated the acyl structure with antibacterial activity. It was found that the presence of unsaturation, cyclopropyl or hydroxyl groups in the acyl moiety of *N*-acyl leucines increased the antibacterial activity and lowered the minimum inhibitory concentration (MIC).

1.1 PLANT DESCRIPTION AND DISTRIBUTION

Vernonia galamensis is an annual shrub, distributed throughout Northern and Central Africa. Different species and subspecies are found in a variety of ecological habitats, ranging from dry forested areas with an yearly rainfall as low as 300 mm to rich forested areas with rainfall of 1850 mm. *Vernonia filisquama* was recorded from several locations in Northern Tanzania. *Vernonia galamensis subspecies galamensis*, includes four varieties which are distributed from West Africa, East to Sudan and Ethiopia, then to Zimbabwe and Mozambique. Vernonia plants known to contain substantial amount of oil includes, *var. galamensis, var. petitiana, var. ethiopica, var. australis*. The distribution of five subspecies of *Vernonia galamensis* found only in East Africa (Uganda, Kenya, and Tanzania), the centre of diversity of species are: *subsp. nairobensis, subsp. afromontana, subsp. gibbosa, subsp. mutomoensis*, and *subsp. lushotoensis.*

1.2 VERNONIA OIL

Generally vernonia seeds contain 40% oil, of which vernolic acid content is 72-80%; palmitic acid, 2.7-3.3%; stearic acid, 2.7-3.9%; oleic acid, 3.6-5.6% and linoleic acid, 12.6-14.0%. One unique advantage of epoxy acid within the triglycerides is that a much greater proportion exists as trivernolin (fig, 1B) than in other oils; hence, it is one of the richest sources of the natural epoxy acid [Ayorinde et al., 1990; Muturi et al.,1993]. Earlier indications are that *Vernonia galamensis* has greater promise agronomically than other potential epoxy oil sources such as *Vernonia anthelmintica, Stokesia laevis*, and *Eurphobia lagascae* species.

The roots of *Vernonia galamensis* plants contain triterpenes and sterols [Mwaura, 1997]. The aerial part contains predominantly sesquiterpene lactones.

$$CH_3-(CH_2)_4-CH-CH-CH_2-CH=CH-(CH_2)_7-COOH$$

(A)

$$CH_2-O-C-(CH_2)_7-CH=CH-CH_2-CH-CH-(CH_2)_4-CH_3$$
$$CH-O-C-(CH_2)_7-CH=CH-CH_2-CH-CH-(CH_2)_4-CH_3$$
$$CH_2-O-C-(CH_2)_7-CH=CH-CH_2-CH-CH-(CH_2)_4-CH_3$$

(B)
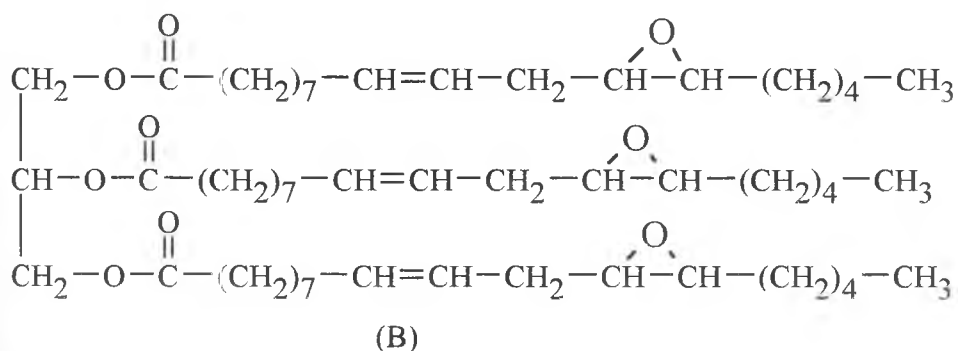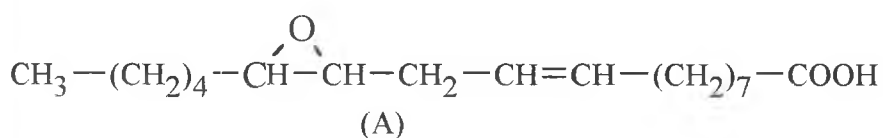
Fig.1. Vernolic acid (A) and trivernolin (B).

The liquid epoxy oil from the seed has properties such as oxirane content (4.1%), viscosity (300 cps at 10°C and 100 cps at 30.5°C) and average molecular weight (926). It is soluble in many organic solvents, diluents and paints and has a homogeneous molecular structure consisting predominantly of identical triglyceride molecules which have three equal vernolic acid residues. Toxicity of vernonia oil should correspond to that of the epoxidized soybean and linseed oils which are industrially produced [Dirlikov et al., 1990].

1.3 OIL EXTRACTION AND PURIFICATION

Vernonia galamensis oil has been extracted from seeds by various methods in different laboratories around the world. This includes soxhlet and cold extraction. The seeds were collected, dried , deactivated and after coarse grinding the oil was extracted with petroleum ether or hexane. It was found that soxhlet extraction yielded somewhat more oil (38%) than stirring ground vernonia seeds with hexane at room temperature (35%) [Grinberg et al., 1994, Kimwomi, 1992].

The extraction of the oil from the seeds on large scale was done by using commercial oil extraction equipment [Ayorinde et al., 1990]. The seeds also contain a lipase which is capable of

hydrolysing the triglycerides. Therefore the seeds were deactivated at conditions of 93.5°C for 90 min. at approximately 15% seed moisture. These conditions were found to be adequate for inactivating the seed lipase and for keeping the free fatty acid content (FFA) of the extracted oil below 1% [Carlson et al., 1985].

The purification of the oil involves degumming with 2- 4% water, followed by bleaching with 2% of a neutral bleaching agent. During the final step of alkali refining, stable emulsions were formed resulting in big losses of the oil. FFA content of the oils averaged 0.6%, and alkali refining reduced this to 0.1%. Charcoal (activated) was the only treatment that removed much color from the oil.

However for laboratory and small-scale pilot-scale processing studies, the soxhlet or temperature extraction of the vernonia seeds is adequate.

1.4 COMPOSITION AND ANALYSIS OF VERNONIA OIL

The components of fatty mixtures containing mono-, di- and triglycerols, fatty acids, fatty amides and cholesterol are analysed and separated by TLC, GC, HPLC, GC/MS and NMR spectroscopic techniques. Bilyk et al. (1991) separated neutral lipids by a rapid unidimensional thin-layer chromatographic (TLC) method, usng two sequential solvent systems of different polarities.

The quantity of trivernolin in vernonia oil was facilitated by High-Performance Liquid Chromatography (HPLC) [Plattner et al., 1977] Trivernolin content was analysed in several samples including three different seed oils. The *Vernonia anthelmintica* seed oils ranged from 39% to 48% trivernolin. The *Euphorbia lagascae* seed oils ranged from 12% to 19% while *Stokesia laevis* seed oils ranged from 1.9% to 49%. The method could potentially be extended for use in analysis of industrial epoxidized oils. Further research done by Carlson et al. (1981) using

quantitative column chromatography, isolated four triglycerides from *Vernonia galamensis* oil in the following relative amounts:

48.2% trivernolin, 37.2% divernoloyl triglycerides, 9.4% monovernoloyl triglycerides and 5.3% normal (unepoxidized) triglycerides. However, gas chromatographic (GC) analysis of the oil by Carlson and Chang found 59.2% trivernolin, 28.1% divernoloyl triglycerides, 9.5% monovernoloyl triglycerides and 3.3% normal triglycerides.

Vernolic acid was found to be predominant at the 1 and 3- glycerol carbons in the vernonia oil using reversed-phase high- performance liquid chromatography with flame ionization detection (RP-HPLC-FID) [Neff et al., 1993]. Isolated triacylglycerols were characterized by proton and carbon nuclear magnetic resonance and capillary gas chromatography of their fatty acid methyl esters.

The crude lipase extract from *Vernonia galamensis* seed was used to synthesize 1, 3-divernoloyl glycerol from vernonia oil in pentane at 40% yield [Ayorinde et al., 1993]. The carbon-13 nuclear magnetic resonance (NMR) spectrum of the 1, 3-divernoloyl glyceride indicated a potential for using carbon-13 NMR spectroscopy in the identification of isomeric diglycerides.

1.5 ANALYSIS AND REACTIONS OF THE EPOXY GROUP

The presence of the epoxy group in vernonia oil makes it very reactive in the presence of acid or base. The oil is an attractive reactive diluent for epoxy or ester formulations [Dirlikov et al. , 1990]. Oxirane oxygen in vernonia oil has been determined by HBr in acetic acid. Oxirane oxygen was determined in salts of epoxy acids and in the presence of amines in epoxy compounds including resins by argentimetric method utilizing hydrogen bromide in acetic acid and silver nitrate, or a completely non-aqueous titration with hydrogen bromide and perchloric acid in acetic acid [Durbetaki, 1958]. Visual end points are employed and the titrations proceed at the speed of aqueous acid base titrations.

Although the above method is rapid and can give good results, the reagent fumes profusely in air and requires special handling and frequent restandardization for accurate analyses. In 1964 Jay developed a better alternative method in which the sample is dissolved in chloroform, mixed with an excess of a soluble quaternary ammonium bromide or iodide and titrated with standard perchloric acid (in acetic acid or dioxane) to a crystal violet end point. The hydrogen bromide (or iodide) generated in situ by the addition of perchloric acid to the quaternary ammonium halide rapidly opens oxirane or aziridine ring:

$$
\begin{array}{ccc}
\underset{\displaystyle HC}{\overset{\displaystyle HC}{\diagdown}} O \quad + \quad HBr & \longrightarrow & -\underset{\displaystyle OH}{\underset{|}{CH}}-\underset{\displaystyle Br}{\underset{|}{CH}}- \\
\end{array}
$$

$$
\begin{array}{ccc}
\underset{\displaystyle H_2C}{\overset{\displaystyle H_2C}{\diagdown}} N- \quad + \quad HI & \longrightarrow & \underset{\displaystyle I}{\underset{|}{CH_2}}-CH_2-NH- \\
\end{array}
$$
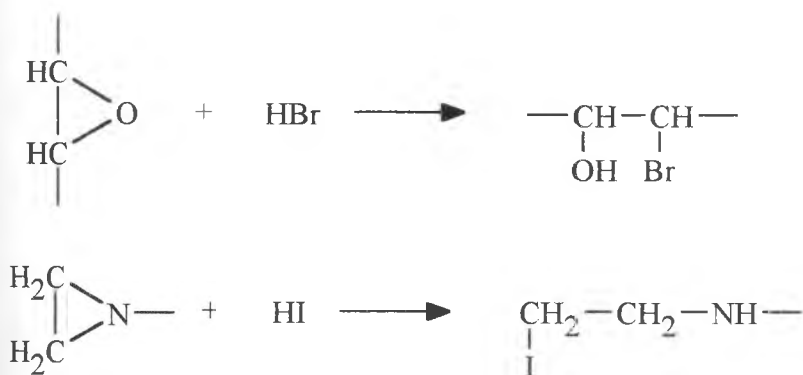
The two methods have been used for the determination of oxirane oxygen in vernonia oil with good results.

The determination of the epoxy group in the vernonia oil was also facilitated by adaptation of the per-iodic acid technique to microsamples [Earle, 1970]. Cleavage of the vernolic acid molecule gives two aldehyde fragments (fig. 2) that can be identified quantitatively by Gas liquid chromatography. An ion exchange resin was then used to convert the epoxy group to a diol inorder to study the geometrical configuration of the acid, which was then compared with known threo- and erythro-9,10-dihydroxystearates by thin layer chromatography or silica gel G plates containing boric acid.

$$CH_3-(CH_2)_4-CH\underset{O}{\diagdown\diagup}CH-CH_2-CH=CH-(CH_2)_7-COOH$$

$$\downarrow \quad \begin{array}{l} 1 \cdot CH_3COOH \\ 2 \cdot KOH \\ 3 \cdot H^+ \end{array}$$

$$CH_3-(CH_2)_4-\underset{\underset{OH}{|}}{CH}-\underset{\underset{OH}{|}}{CH}-CH_2-CH=CH-(CH_2)_7-COOH$$

$$\downarrow \quad HIO_4$$

$$CH_3(CH_2)_4-\underset{\underset{O}{\|}}{CH} \quad + \quad O=CHCH_2CH=CH(CH_2)_7COOH$$
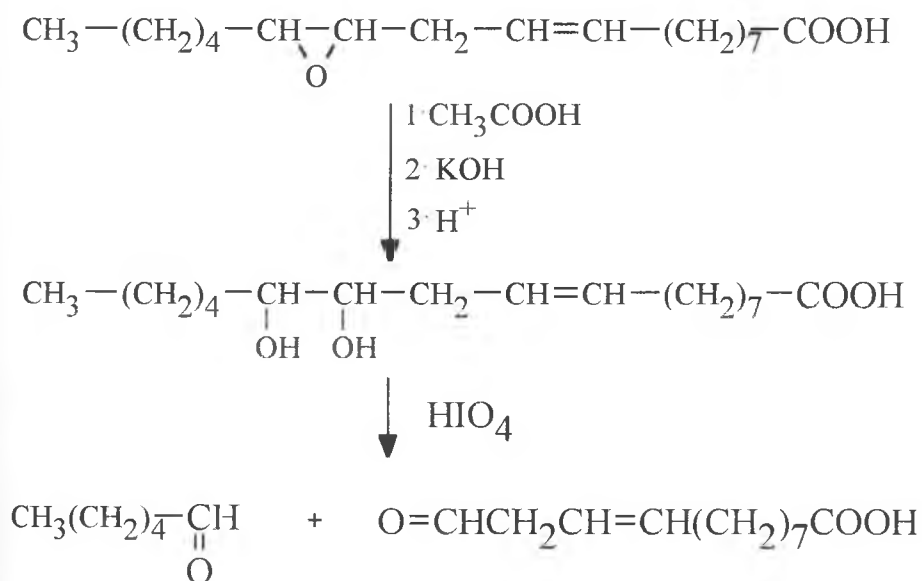
Fig. 2: Periodic acid cleavage of vernolic acid

The determination of ring opening for a number of unsymmetrical epoxides has shown that the attack appears to be largely at the primary carbon atom. Therefore, the $S_N{}^2$ reaction mechanism which takes place in the cause of the ring openig process seem to be dictated by both steric and electronic effects [Vanderwerf et al., 1954].

$$R-CH\underset{O}{\diagdown\diagup}CH_2 + :Nu \longrightarrow R-\underset{\underset{OH}{|}}{CH}-CH_2-Nu$$

Where Nu = nucleophile

1.6 INTERPENETRATING POLYMER NETWORKS

Primary and secondary amines are conventionally used as curing agents for epoxy polymers. Resins with a hydrophillic character are usually prepared by the following main reaction:

$$R-NH_2 \quad + \quad -CH\underset{O}{\diagdown\diagup}CH- \quad \longrightarrow \quad -\underset{\underset{OH}{|}}{CH}-\underset{\underset{NHR}{|}}{CH}-$$
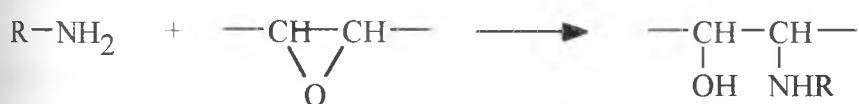
The multiple chemical functionality of vernonia oil makes it possible to react with many chemical reagents yielding polyurethane or polyester polymer networks. When such polymer networks are

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combined with a second polymer in network form, an interpenetrating polymer network (IPN) is produced [Sperling et al., 1983]. Such IPN form impact-resistant plastics or reinforced elastomers depending on overall composition and phase continuity.

A toughened elastomer was synthesized by reacting vernonia oil with octanedioic acid (derived from vernonia oil), and cross-linking the pre-polymer in the immediate presence of cross-linked polystyrene prepared in situ [Afolabi et al., 1989]. The progress of the reaction was followed by monitoring the generation of hydroxyl groups using infra-red spectroscopy. At 140°C, the reaction between vernonia oil and suberic acid is primarily that between carboxylic acid groups of suberic acid and epoxy groups of vernonia oil (fig.3):

$$
\begin{array}{c}
\overset{\displaystyle O}{\overset{\displaystyle \diagup\!\!\diagdown}{-CH\!-\!CH\!-\!-}} \\[2em]
\overset{\displaystyle O}{\overset{\displaystyle \diagup\!\!\diagdown}{-CH\!-\!CH\!-\!-}}
\end{array}
\;+\;
\begin{array}{c}
COOH \\
| \\
(CH_2)_6 \\
| \\
COOH
\end{array}
\;\longrightarrow\;
\begin{array}{c}
OH \\
| \\
-CH\!-\!CH\!- \\
| \\
O \\
| \\
C\!=\!O \\
| \\
(CH_2)_6 \\
| \\
C\!=\!O \\
OH\quad | \\
|\quad\;\; O \\
--CH\!-\!CH\!-
\end{array}
$$
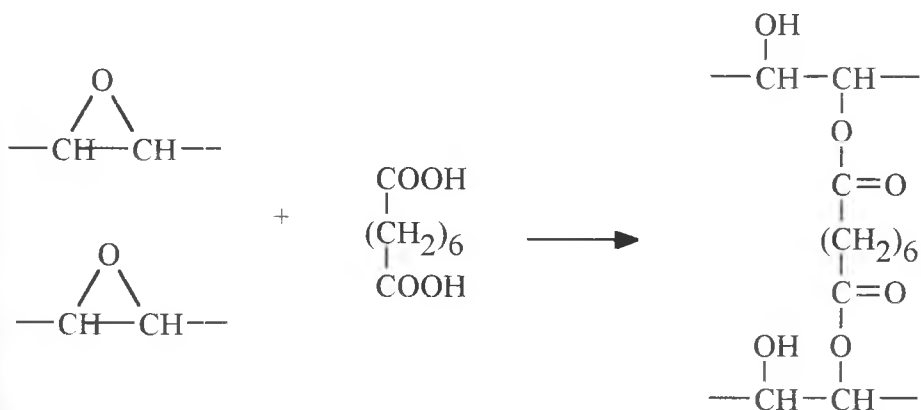
FIG.3 Reaction scheme for vernonia oil-suberic acid prepolymer.

Cross-linking by the suberic acid can occur intramolecularly as well as intermolecularly. Future studies will include the use of various dibasic acids and the material characterization of the resulting polymers.

An oil bearing either hydroxyl or epoxide functionality may be cross-linked to form a soft elastomer in the presence of another monomer or network to form an interpenetrating polymer network (IPN), or in the presence of a linear polymer to form a semi-IPN [Barrett et al., 1993]. Polymerization and characterization of naturally functionalized triglyceride oils has been reported

with emphasis on the distribution and effect of nontrifunctional triglycerides on elastomer properties, such as toughness and mechanical properties.

The vernonia oil polyester elastomer may also be prepared by reaction with dibasic acids, themselves derived from the oil, forming a natural product elastomer. Fernandez et al. (1983, 1986) have produced vernonia/polystyrene IPNs by using sebacic acid as the vernonia oil cross linker. These IPNs had properties ranging from reinforced elastomer to toughened plastic, depending on the overall composition.

1.7 DIBASIC ACIDS FROM VERNONIA OIL

Vernonia oil is a good source of dibasic acids including butanedioic acid, pentanedioic acid, hexanedioic acid (adipic), heptanedioic acid (pimelic), octanedioic acid (suberic), nonanedioic acid (azelaic), decanedioic acid (sebacic) and undecanedioic acid [Ayorinde et al., 1988, 1989). These acids were previously synthesized from petrochemical based products,*Vernonia galamensis* oil therefore can serve as an alternative because of its cheap availability. For example, dodecanedioic acid was synthesized according to the scheme below:
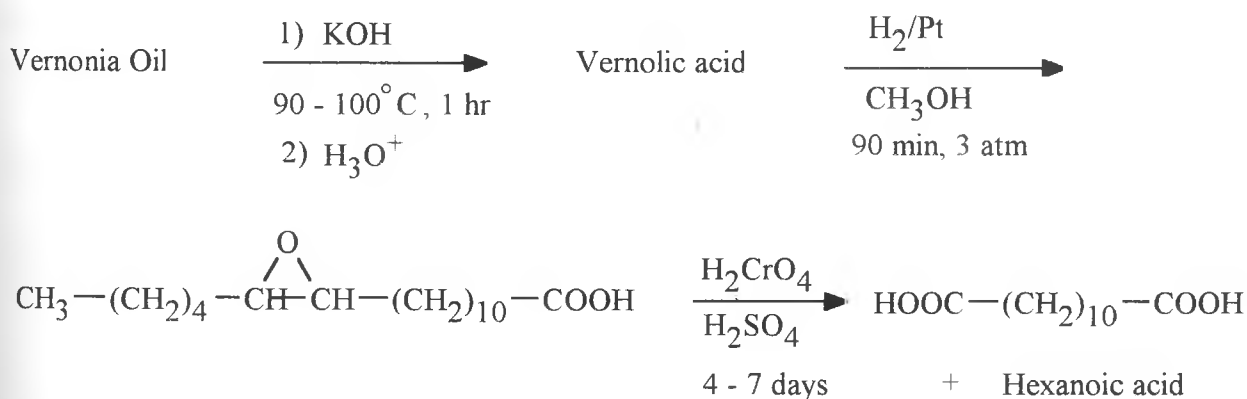
Vernonia Oil $\xrightarrow[\substack{90 - 100^\circ C,\ 1\ hr \\ 2)\ H_3O^+}]{1)\ KOH}$ Vernolic acid $\xrightarrow[\substack{CH_3OH \\ 90\ min,\ 3\ atm}]{H_2/Pt}$

$$CH_3-(CH_2)_4-\overset{\overset{\displaystyle O}{\diagup\diagdown}}{CH-CH}-(CH_2)_{10}-COOH \xrightarrow[\substack{H_2SO_4 \\ 4 - 7\ days}]{H_2CrO_4} HOOC-(CH_2)_{10}-COOH$$

$$+ \quad \text{Hexanoic acid}$$

Nonanedioic acid is obtained from triglyceride oils and fatty acids containing unsaturation between C_9- C_{10}. On the other hand castor oil is the only triglyceride oil that has been used as a

commercial source of octanedioic (suberic) acid. Castor oil (fig. 4), is structurally related to trivernolin (the major component of vernonia oil). Castor oil has received much attention, because of its status as a large-scale commercial product [Sperling et al., 1991]. Castor oil from castor beans is the triglyceride of ricinoleic acid, with 83.6% to 90% of all acid residues being ricinoleic acid. Castor oil is routinely used in paints, adhesives and urethane foams. Through alkali pyrolysis, castor oil is also a major source of dibasic acids such as decanedioic acid (sebacic acid), a major component of nylon. The above dibasic acids and their derivatives are used in the manufacture of polyurethanes, polyamides, alkyd resins, plasticizers, elastomers (synthetic rubber), lubricants and hydraulic fluids.

$$CH_2-O-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_7-CH=CH-CH_2-\overset{\overset{\displaystyle OH}{|}}{CH}-(CH_2)_5-CH_3$$
$$CH-O-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_7-CH=CH-CH_2-\overset{\overset{\displaystyle OH}{|}}{CH}-(CH_2)_5-CH_3$$
$$CH_2-O-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_7-CH=CH-CH_2-\overset{\overset{\displaystyle OH}{|}}{CH}-(CH_2)_5-CH_3$$
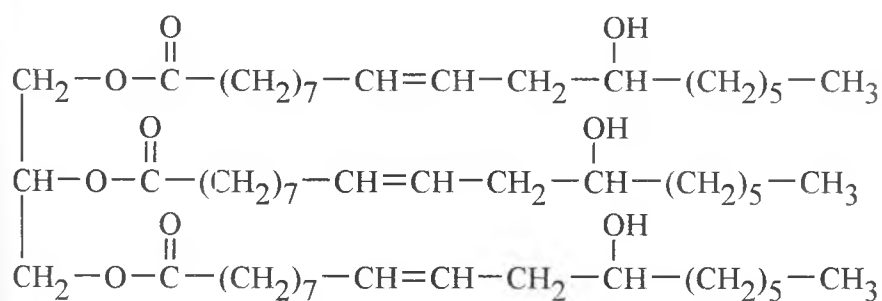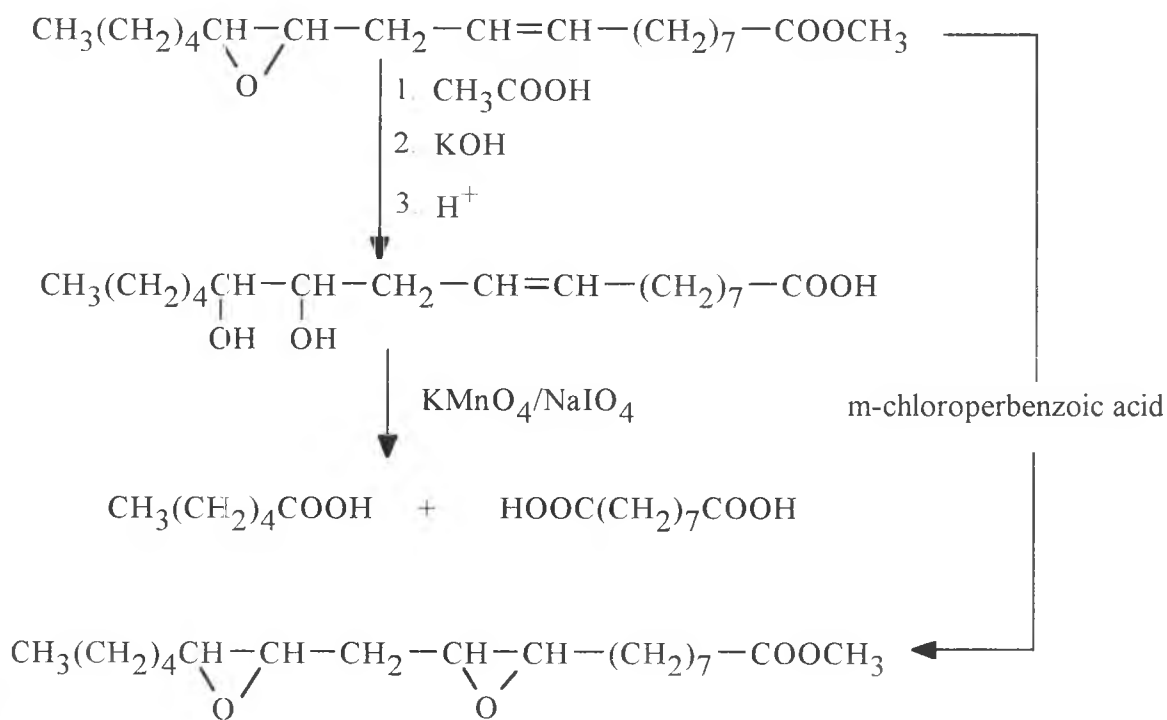
Fig. 4. Chemical structure of triricinolein, major constituent of castor oil.

Siddiqi and co-workers (1984) also synthesized nonanedioic acid (scheme 1, page 12) from *Vernonia volkameraefolia* seed oil which has a vernolic acid content of 63.5%. Therefore it is evident that vernonia oil can compete with other existing oils on the market.

$$CH_3(CH_2)_4CH\!-\!CH\!-\!CH_2\!-\!CH\!=\!CH\!-\!(CH_2)_7\!-\!COOCH_3$$

(epoxy ring at O)

1. CH_3COOH

2. KOH

3. H^+

$$CH_3(CH_2)_4CH\!-\!CH\!-\!CH_2\!-\!CH\!=\!CH\!-\!(CH_2)_7\!-\!COOH$$
$$\quad\quad\quad\quad OH\quad OH$$

$KMnO_4/NaIO_4$

m-chloroperbenzoic acid

$$CH_3(CH_2)_4COOH\quad+\quad HOOC(CH_2)_7COOH$$

$$CH_3(CH_2)_4CH\!-\!CH\!-\!CH_2\!-\!CH\!-\!CH\!-\!(CH_2)_7\!-\!COOCH_3$$

Scheme 1: Derivatives from vernonia oil

1.8 AMINATION OF VERNONIA OIL.

Vernonia oil undergoes hydrolysis and transesterification reactions. The oil reacts with alcohols (methanol, ethanol, 1-propanol and 2-propanol), under acidic conditions, to result in transesterification as well as epoxy ring opening in all cases [Bryant et al., 1992]. It was found that the major products, the hydroxy alkoxy fatty esters, constitute approximately 80% of the product mixtures of which the 12-hydroxy-13-alkoxy isomers were the major constituents. Alcoholysis with butanol resulted in a poor yield of the hydroxy butoxy esters.

Transesterification of vernonia oil with methanol or other higher molecular weight monofunctional alcohols affords preparation of very low viscosity diluents. For example methyl vernolate (fig. 5) has been prepared in quantitative yield:
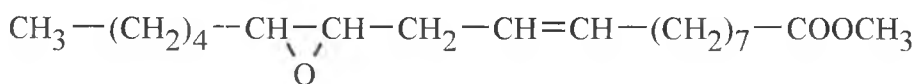
$$CH_3-(CH_2)_4--CH-CH-CH_2-CH=CH-(CH_2)_7-COOCH_3$$
$$O$$

Fig. 5 Methyl vernolate

Therefore, the oil constitutes a viable synthetic starting material for hydroxy alkoxy acids and esters due to its high vernolic acid content.

1.9 SYNTHESIS OF FATTY AMIDES

Fatty amides, which are important chemical intermediates, have been synthesized by several methods under different conditions. One of the method involves the preparation of the fatty amides or diamides from the reaction of fatty acids with amines at relatively high temperatures. However, the yields of the products obtained are low hence necessitating the development of more direct methods which give better yields. Inorder to reduce energy costs, Feairheller et al. (1994) synthesized fatty alkanolamides, fatty diamides and fatty arylamides directly from triglycerides (tallow and tripalmitin) and primary amines at low temperatures.

With the discovery of vernonia oil epoxy amides have been synthesized to obtain derivatives of higher added value. Bisamides were obtained by reacting the natural epoxy triglyceride (vernonia oil) with aliphatic diamines with the general formula $R-CO-NH-(CH_2)_nNH-CO-R$

$$\text{Where R} = CH_3(CH_2)_4CH-CH-CH_2-CH=CH-(CH_2)_7-$$
$$O$$
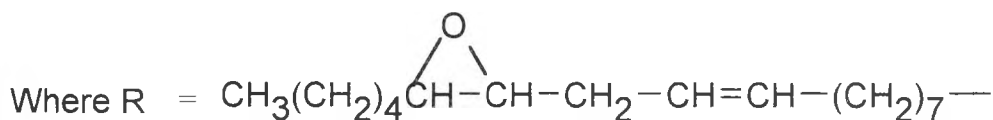
[Wawira, 1996; Grinberg et al., 1994].

The fatty bisamides can be used as new reactive difunctional epoxy monomers.

In the reaction of vernonia oil with an aromatic diamine the expected opening of the epoxy group took place Cross- linked rubber like elastomers with improved thermoresistant properties

were obtained in the presence of an acid catalyst. It is known that aromatic amines are less basic than the aliphatic amines (pKa 4.5 - 5.0); therefore, they are less reactive than aliphatic amines.

The reaction of *Vernonia galamensis* oil with butanamine, pentanamine and hexanamine afforded high yields of epoxidized secondary amides under reflux with amines as solvents, reflux with hexane as solvent and room temperature with the amines as solvents [Bryant et al., 1993]. Reactions with amines as refluxing solvents were completed first, followed by those with hexane, while room temperature reactions took longer.

The amidation of vernonia oil resulted in the formation of N-alkylvernolamides (fig. 6):

$$CH_3-(CH_2)_4-CH-CH-CH_2-CH=CH-CH_2-(CH_2)_5-CH_2-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-(CH_2)_n-CH_3$$

where n = 3, 4, 5

Fig. 6: N- alkyl vernolamides from vernonia oil

The amine reactivity was directly correlated to chain length The higher homologues react more readily with the triglycerides due to their greater lipophilic nature. It was further conceived that the greater nucleophilicity of the higher amine homologues could be attributed to a greater electron donating effect of the alkyl groups due to hyperconjugation. However, the molar ratios of vernonia oil to the amine, temperature, reaction period and solvents influence the aminolysis of the triglycerides [Wawira, 1996].

Although these reactions are all nucleophilic in nature, they do not always result in epoxy ring opening implying that the epoxy group which is located on carbons 6 and 7 from the end of the alkyl chain is substantially hindered. It is, therefore relatively stable under alkaline conditions

However, it is prone to ring opening under acidic conditions. The amines were used in excess and serve as solvent, reagent and perhaps as catalyst.

The chemical form of the fat moiety or amine has a significant effect on the rate of amidation. For example, the tallow amidation with diethylenetriamine was completed faster than that of methyl tallowate. When the tallow was reacted with aniline, only a 7% yield of tallowanilide was obtained; however, reaction with benzylamine yielded 90% of N-benzyltallowamide. The poor yield of tallowanilide was due to the structure of aniline where the non-bonded pair of electrons which is responsible for the nucleophilicity of amino groups is dispersed over the aromatic ring in the aniline molecule; hence, not available for reactions not to attack. For benzylamine, the methylene group of the amine prevents the delocalization of the non-bonded electron pair into the aromatic ring and; hence, it is always available to react.

The butylamide of ricinoleic acid [N-(*n*-butyl)-12-hydroxy-(9Z)-octadecenamide] was prepared from a neat mixture of castor oil and *n*-butylamine (fatty ester/ amine molar ratio, 1:1.3) by Piazza and co-workers (1993) . No catalyst was required and no solvent was present. High product yields were achieved at 45°C and 65°C in 48 and 20 hours, respectively. The reaction was inhibited by the addition of dioxane and trimethylpentane, but not by water. This demonstrates the ease with which secondary fatty amides can be prepared from an oil that consists primarily of the glycerol esters of hydroxylated fatty acids.

Fatty alkanolamides find their application in detergents, shampoos, lubricants, cosmetics foam control agents and water repellents. Fatty diamides are used as lubricants, antistatic agents, release agents, corrosion inhibitors and water repellents, while fatty anilides are used as herbicides, fungicides, insecticides, defoliants and bactericides. Epoxy compounds have assumed commercial importance in recent years with the advent of epoxy resins, epoxy plasticizers, epoxy stabilizers, and epoxy insecticides

1.10 OBJECTIVES AND SIGNIFICANCE OF THE STUDY

From the above considerations, vernonia oil has a great potential due to the presence of reactive functional groups The double bond can undergo addition, ozonolysis, epoxidation, hydrogenation and polymerization reactions while the oxirane group undergoes hydration, alcoholysis, amination and polymerization reactions. Finally, hydrolysis, amidation and transesterification at the ester group results in the formation of fatty acids, fatty amides and alkylesters of vernolic acid.

Appropriate conditions for extraction, purification and synthesis of derivatives are necessary due to the reactive nature of the naturally epoxidized triglyceride oil. This leads to finding optimum conditions (temperature, reaction period, molar ratios of reactants and solvents) to ensure that high yields of the derivatives are obtained. The chemical form of the amine moiety has a significant effect on amidation, hence it is possible to study amine reactivity with vernonia oil.

Recent studies focused on characterization of vernonia oil and its applications although the trend has shifted to its derivatives and their applications The amides have been prepared from alkylamines and hydroxyamines. The primary aminoamides have also been synthesized. The study aims at synthesizing aminoamides containing tertiary amino groups and studying their application as antifungal agents.

The possibility of these compounds finding application as polymeric resins and chelating agents is under investigation. For example, N-N'-ethylenebis(vernolamide) can act as a bidentate ligand to offer two places of attachment to form a 5- ring system with metal cations which is quite stable. Likewise the amides and diamides can be used as polymeric resins to exchange hydrogen ions in solution by forming quaternary ammonium ions.

The opening of the epoxy group in epoxy amides leads to the formation of diols, hydroxyesters and hydroxyalkoxy compounds. Reacting the epoxy group with a carboxylic acid such as acrylic acid may lead to the elongation of the chain and form polymers.

Finally, earlier indications were that some fatty amides have been used as fungicides, herbicides, insecticides, defoliants and bactericides. The derivatives from vernonia oil may find application as bactericides and fungicides.

The objectives of the research were :

1. To extract and isolate vernonia oil

2. To synthesize and characterize vernolamides from vernonia oil

3. To study the application of the vernolamides as bactericides and fungicides by testing their biological activity, and

4. To perform reactions on the derivatives with respect to the epoxy group.

CHAPTER TWO

EXPERIMENTAL

2.0 SEED TEMPERING (LIPASE-INACTIVATION) OF VERNONIA GALAMENSIS SEEDS

The vernonia seeds, were collected from Kabete and Kibwezi areas where they are currently being grown. The seeds were air-dried and subjected to lipase inactivation in an oven maintained at 95 - 100°C for 90 minutes. The seeds were kept moist by sprinkling with water at 30 minute-interval during tempering, subsequently removed from the oven and air-dried. The dried, lipase-inactivated seeds were then crushed using a laboratory Wiley Mill, Model No. 2.

2.1 OIL EXTRACTION AND PURIFICATION

All extractions were with commercial hexane. Three soxhlet extractors were used. Other laboratory extractions were carried out with 20 g, 100 g, 200 g and 900 g of crushed vernonia seeds depending on the size of the soxhlet thimble. Contact times for individual solvent fractions ranged from 1 hr (20 g scale) to 6 hr. Solvent fractions were combined or kept separate depending on the purpose of the experiment.

Dry and powdered seeds of *Vernonia galamensis* (550 g) were extracted in a soxhlet with commercial hexane (2.5 l). Flakes were contained in cotton cloth bags. Hexane was removed by a rotary evaporator to obtain the slightly dark colored crude oil which was weighed.

The crude oil was degummed by stirring the oil with distilled water at the ratio of 21:1 at 50°C for 1 hour on a magnetic stirrer, followed by centrifugation for 3 hours. The degummed oil was then decolourized with activated charcoal at levels of 2% and 5% by weight. This was done

by mixing the oil with the activated charcoal and stirring at 60°C for 1 hour on a magnetic stirrer followed by filtration. It was found that 5% gave the best results.

The degummed and charcoal-treated oil was bleached with fuller's earth (1%) by mixing on a rotary evaporator at 60°C for 30 minutes and filtered. Then finally, alkali-refining was done by adding 2N NaOH in the ratio 34:1 with stirring for 30 minutes. The oil was dried on a rotary evaporator at 60°C for 30 minutes, then stored in a refrigerator at 4°C for further analysis. The yield was 126.5 g (23%). The same procedure was repeated to extract other oil samples. Infrared (IR) (cm^{-1}) in nujol: 1760 (ester group); 850, 840 (epoxy group).

2.2 EQUIPMENT

Thin layer chromatographic analyses (TLC) were performed on commercial pre-coated plates (0.25 mm silica gel 60, MN). Developing solvents were hexane:diethylether (70:30 or 60:40 v/v) and chloroform: methanol (90:10). Visualization with iodine was carried out by placing the developed, dried layer in a jar containing crystalline iodine. Column chromatography was run on silica gel 60 (0.063 - 0.100 mm, E. Merck) packed in a 2.0 x 50 cm glass column. Reaction mixtures were separated on silica gel by elution with chloroform. Infrared (IR) spectra of the solids in nujol mull were recorded on Pye Unicam -SP3-300 IR spectrophotometer. Electron impact ionization (EI) mass spectral data was obtained by a TSQ 70 (FINNIGAN MAT) spectrometer. ^{1}H- and ^{13}C-NMR spectra were obtained with a Bruker (AC-250) spectrometer in CDCl$_3$ solution (TMS). ^{1}H-NMR was recorded at 250 MHz while ^{13}C-NMR spectra were recorded at 50 MHz. The melting points of the derivatives were determined using the GALLENKAMP melting point apparatus.

2.3   REACTIONS OF VERNONIA OIL WITH AMINES

2.3.1 Synthesis of N-2-(1-pyrrolidino)ethylvernolamide

Vernonia oil (2.70 g, 2.92 mmole) based on a molecular weight of 926 and 1-(2-aminoethyl)pyrrolidine (1 g, 8.76 mmole) were added to a round-bottomed flask (25 mL) equipped with a reflux condenser and a magnetic stirrer. The reaction was allowed to proceed at 50°C for 15 hours and the formation of the product monitored by TLC. After cooling, the reaction mixture was dissolved in 10 mL of chloroform and transferred into a 100 mL separatory funnel, to which 10 mL of water was added. Then it was shaken and allowed to partition. After removing and discarding the aqueouns layer, the organic portion was washed five times with 10 mL portions of water to remove any unreacted amine. The organic portion was transferred into a 100 mL round-bottomed flask and the chloroform evaporated under vacuum. Hexane was added and placed in ice to precipitate the crude yellow epoxy amide, which was filtered and washed several times with cold hexane to remove any unreacted oil.

The product was dried in air to afford 1.55 g (45%) of an amorphous yellow compound, with a melting point of 42 - 44°C. Infrared (IR) (cm^{-1}) in nujol: 3300 (NH), 1640 (carbonyl group),1550, 845 and 825 (epoxy group). Mass-spectral data for the isolated N-2-(1-pyrrolidine)ethylvernolamide exhibited major peaks at m/z 84 (100), 156 (3.1), 169 (7.6), 225 (21), 279 (22.3), 321 (46.6), 363 (2.3) and 393.4 (9.2). ^{1}H-NMR (CDCl$_3$) δ ppm: 6.30 (NH), 5.42 -5.65 (CH=CH), 3.39- 3.48 (CH$_2$N), 2.97 - 3.02 (epoxy protons), 1.32 - 1.89 (complex, -(CH$_2$)$_n$-) and 0.9 (CH$_3$); ^{13}C-NMR (CDCl$_3$) δ ppm: 173.25 (C=O), 123.86-132.57 (CH=CH) and 56.54-57.21 (epoxy carbons).

2.3.2 Synthesis of N-3-(4-morpholino)propylvernolamide

The above procedure for the synthesis of N-2-(1-pyrrolidino)ethylvernolamide was repeated where vernonia oil (2.14 g, 2.31 mmol) and 4-(3-aminopropyl)morpholine (1 g, 6.93 mmol) were used. A white, shiny, soft and low density amorphous compound was obtained with a melting point of 50-52°C after drying in air. The yield was 0.59 g (20%). Infrared (IR) (cm^{-1}) in nujol: 3300 (NH), 1640 (carbonyl group), 1550, 840 and 825 (epoxy group). Mass spectral data for the isolated amide exhibited major peaks at m/z 43 (18.7), 57 (8.9), 86 (16.7), 100 (100), 114 (12.4), 128 (8 9), 143 (3.7), 167 (6.0), 171 (3 1) 186 (5.1), 199 (14.6), 213 (2.7), 236 (7.8), 279 (7.8), 295 (2 4), 309 (4.1), 336 (10.6), 351 (27.2), 365 (0.6), 379 (2.6), 423 (9.3) and 424 (10.0). ^{1}H-NMR (CDCl$_3$) δ ppm: 6.85 (NH), 5 40 - 5.60 (CH=CH), 3.80 (CH$_2$O), 3.39- 3.48 (CH$_2$N), 2.90 - 3.0 (epoxy protons), 1.30 - 1.80 (complex, -(CH$_2$)$_n$-) and 0.9 (CH$_3$); ^{13}C-NMR (CDCl$_3$) δ ppm 172.89 (C=O), 123.92-132.53 (CH=CH) and 56.53-57 87 (epoxy carbons).

2.3.3 Synthesis of N-3-[1-(2-oxopyrrolidino)]propylvernolamide

A mixture of 2.70 g (2.92 mmol) of vernonia oil and 1.25 g (8.79 mmol) of 1-(3-aminopropyl) -2-pyrrolidinone was stirred with a magnetic stirrer at 50°C for 17 hours. The reaction mixture was monitored by TLC Then the reaction mixture was preadsorbed on silica gel and poured as a powder on a short column packed with silica gel to trap excess amine. It was eluted with chloroform and TLC analysis of the effluent collected confirmed the absence of the amine

The chloroform was stripped off by a rotavapor under vacuum and the mixture dissolved in hexane Then allowed to cool for crystallisation to occur A white compound crystallised out, which was filtered and washed several times with hexane to remove unreacted oil. The product,

which was white amorphous, thin, shiny sheets, 0.74 g (20% yield) had a melting point of 52°C.

Infrared (IR) (cm^{-1}) in nujol: 3300, 1680, 1645, 1550, 840 and 825.

The mass spectrum of the isolated epoxy amide exhibited major peaks at m/z 98 (46.4), 112 (56.4), 126 (100), 143 (26.5), 169 (4.1), 184 (7.2), 197 (9.0), 211 (0.7), 253 (1.4), 279 (1.0), 307 (8.8), 349 (2.9) and 420 (0.4). ^{1}H-NMR (CDCl$_3$) δ ppm: 6.71 (NH), 5.30 - 5.58 (CH=CH), 3.16 - 3.43 (CH$_2$N), 2.90 - 2.92 (epoxy protons), 1.30 - 1.70 (complex, -(CH$_2$)$_n$-) and 0.91(CH$_3$); ^{13}C-NMR (CDCl$_3$) δ ppm: 123.89-132.68 (CH=CH) and 56.60-57.25 (epoxy carbons).

2.3.4   Synthesis of N-3-(1-imidazole)propylvernolamide

Vernonia oil (3.70 g, 4.0 mmole) and 1-(3-aminopropyl)imidazole (1.5 g, 12 mmole) were added to a round-bottomed flask (25 mL) containing 6 mL of dimethylformamide. The reaction was allowed to proceed on a magnetic stirrer at 50°C for 48 hours. After cooling, a compound crystallised out in the process which was filtered and washed five times with hexane to remove the unreacted oil. The product was then dried in air to afford 0.73 g (15%) of an amorphous yellow compound with a melting point of 48-50°C. Infrared (IR) (cm^{-1}) in nujol: 1650 (carbonyl group), 1550, 840 and 825 (epoxy group).

2.4   SYNTHESIS OF DIHYDROXY AND HYDROXY ESTERS FROM VERNOLAMIDES

2.4.1   DIHYDROXY AMIDES

A mixture of 0.20 g (0.51 mmole) of N-2-(1-pyrrolidino)ethylvernolamide and 5 mL of 1 M sulphuric acid were added to a round-bottomed flask. Then stirred continuously on a magnetic stirrer for 1 hour and the contents of the reaction mixture poured into a 100 mL separatory funnel where two layers formed. The lower layer (brown oil) was removed and dissolved in 10 mL of

ethanol and dried over calcium chloride. The ethanol was removed in a rotary evaporator to afford 0.12 g (57%) of a brown oil, which was insoluble in hexane, chloroform and ether.

2.4.2 Acetylation of N-2-(1-pyrrolidino)ethylvernolamide

In a 25 mL round-bottomed flask equipped with a magnetic stirrer, 5 mL of glacial acetic acid was added to 0.50 g (1.28 mmol) of N-2-(1-pyrrolidino)ethylvernolamide. The mixture was stirred continuously at room temperature for 2 hours, then transferred to a 100 mL separatory funnel to which 20 mL of hexane had been added. The hexane layer was removed and discarded. Then washed with aqueous sodium bicarbonate to remove excess acetic acid, dried over anhydrous calcium chloride and 0.30 g (51%) of a yellow oil obtained.

2.4.3 Confirmatory tests for the dihydroxy derivatives

a) With metallic sodium

A small sample of the product was put in a test tube and a piece of metallic sodium from a bottle stored in oil was dried quickly on a small piece of filter paper and put into the test tube. There was a steady evolution of gas bubbles, which could only be hydrogen.

b) With Acetyl Chloride

A few drops of the product were slowly and cautiously added to a few drops of acetyl chloride in a dry test tube. There was a vigorous evolution of hydrogen chloride gas and heat.

c) The Hydroxamic Acid Test

A few drops of the product in (b) above were added to about 0.5 ml of a solution of hydroxylamine hydrochloride in methanol (5%) and followed with a dilute solution of methanolic potassium hydroxide until the solution was just alkaline (tested with litmus). The solution was boiled for a minute, cooled and just acidified with dilute hydrochloric acid.

A few drops of ferric chloride solution were added. A red color was observed indicating the presence of hydroxamic acid, hence the product formed was an ester.

2.5 ANTIMICROBIAL TESTS

The epoxy amides were tested for antimicrobial activity. The amines: 1-(2-aminoethyl)pyrrolidine, 4-(3-aminopropyl)morpholine, 1-(3-aminopropyl)-2-pyrrolidinone and vernonia oil were also tested.

2.5.1 Antibacterial properties

Nutrient agar (composition : 31.4 g/L) was sterilised at 121°C for 15 minutes. Then cooled before pouring on petri dishes which had been sterilized in an autoclave. Portions of *Staphylococcus aureus, Bacillus subtilis* and *Escherichia* coli were spread on the petri dishes of nutrient agar. After drying the petri dishes in a sterile hood (Lamina Air flow, gelaine HF 72, flow laboratories from Italy) for 15 to 30 minutes, blank sterile filter paper discs (5 mm diameter) were placed with sterile forceps.

A solution of the sample (4 μL, 2 μL, and 1μL of 25 mg/mL chloroform solution equivalent to 100 μg, 50 μg, and 25μg of the respective samples) were spotted at the centre of the paper discs. The petri dishes were then placed in the incubator overnight at 37°C. The diameter of the inhibition zone was obtained from the average of two determinations in each case.

2.5.2 Antifungal properties

Media preparation

The media was prepared by weighing 39 grams of potato dextrose agar (composition: potato extract 4.0 g/L, dextrose 20.0 g/L and agar 15.0 g/L) and dispersed in 1 litre of deionised water. The agar mixture was left standing for 10 minutes and swirled to mix and sterilised at 121°C for 15 minutes. It was cooled to 47°C and mixed well before pouring on sterilised petri dishes.

Inoculation

Portions of *Trichophyton mentagrophyte*, *Microsporum gypseum*, *Candida albicans* and *Saccharomyces cerevisiae* were spread on the petri dishes of the potato dextrose agar. After drying the petri dishes in a sterile hood for 15 to 30 minutes, sterile filter paper discs (5 mm diameter) were placed on the petri dishes with sterile forceps. The samples were spotted in solution form (4 µl, 2 µl and 1µl of 25 mg/mL chloroform solution). In addition, a blank disc with only chloroform was used as a control. The petri dishes were placed in the incubator at 37°C for four days. The diameter of the inhibition zone was obtained from the average of three determinations in each case.

CHAPTER THREE

RESULTS AND DISCUSSION

3.0 OIL EXTRACTION

The crude oil extracted ranged from 19- 31 % while a big loss of the oil was incurred during the refining process, resulting in 15- 27% of the refined oil. This was attributed to the decolourising step where the oil was treated with charcoal (5% w/w, charcoal to oil). The percentage of epoxy oil in the seeds depends on the variety of *Vernonia galamensis* as previously reported in the literature [Wawira, 1996].

Table 1 Extraction of vernonia oil (Soxhlet)

Crushed seeds (g)	Extraction yield, %	Refined oil, %
550	26.4	23.0
796	20.4	15.0
905	25.3	24.0
900	27.9	24.5
705	30.7	27.0
825	23.1	18.8
902	28.9	23.5
232	20.0	18.7
200	19.9	17.7
100	20.3	18.1
100	25.3	23.7
20	19.2	17.7
20	19.1	17.1

The IR spectrum cofirmed the presence of the ester group (1760 cm^{-1}) and the epoxy group (850, 840 cm^{-1}) in the oil. Therefore, the epoxy group was stable during degumming and alkali refining of the oil where it was in contact with water and sodium hydroxide respectively.

3.1 EXPERIMENTAL RESULTS FROM SYNTHESIS OF EPOXY AMIDES

Table 2 Experimental results from synthesis of epoxy amides

Epoxy amides	Reaction Time (hrs)	R$_F$ Value	% Yield	M.P.(°C)
N-2-(1-pyrrolidino)ethylvernolamide	16	0.4	45	42- 44
N-3-(4-morpholino)propylvernolamide	18	0.63	20	50-52
N-3-[1-(2-oxopyrrolidino)]propyl-vernolamide	17	0.7	20	52

Purity of the amides was confirmed by obtaining a single spot in each case on thin-layer chromatography plates, while the amines did not move but remained at the origin when using chloroform: methanol (90:10) as the developing solvent system It was observed that the reactivity of 1-(2-aminoethyl)pyrrolidine with vernonia oil was faster, resulting in a higher yield (45%) of N-2-(1-pyrrolidino)ethylvernolamide, as compared to the other two amides.

3.2 Reaction of vernonia oil with primary amines

The reactions did not go to completion at room temperature due to the formation of semi-solid reaction mixtures. However, the reactions went to completion at 50°C The amines were used in excess in molar ratios of 1:3 (vernonia oil:amine). The amines acted as reactants and solvents.in the reactions.

3.2.1 Formation of N-2-(1-pyrrolidino)ethylvernolamide

The reaction of vernonia oil with 1-(2-aminoethyl)pyrrolidine yielded N-2-(1-pyrrolidino)-ethylvernolamide. The disappearance of the triglycerides (vernonia oil) and formation of the product was followed using TLC chromatography.

Vernonia oil $+$ $NH_2-CH_2CH_2-N$⟨pyrrolidine⟩

$$CH_3(CH_2)_4-CH-CHCH_2CH=CH(CH_2)_7CONHCH_2CH_2N$$
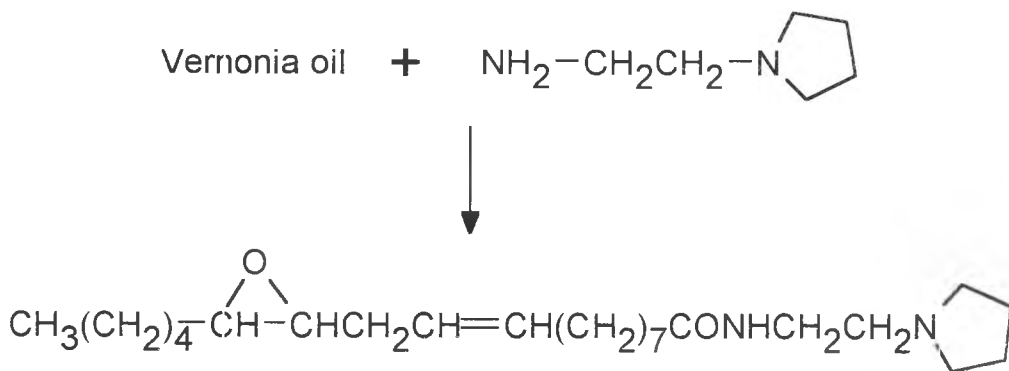
Scheme 2: Formation of N-2-(1-pyrrolidino)ethylvernolamide

The infrared spectra for the epoxy fatty amide showed sharp absorptions at 3300 cm^{-1} typical of secondary amides. Absorptions at 1640 cm^{-1} and 1550 cm^{-1} suggested the carbonyl of the amide group, indicating that the amminolysis reaction had taken place at the ester bond, giving an amide as a product. The epoxy absorption was still present at 825 cm^{-1} and 845 cm^{-1}

The M+1 peak was observed at m/z 393.4 in the EI MS while the base peak at m/z 84.1 was due the alpha cleavage to the nitrogen. The McLafferty rearrangement gave a fragment ion at m/z 156.1 and beta cleavage to the carbonyl group gave a peak at m/z 169. Cleavage between C7 and C8 (allylic cleavage) resulted in a fragment at m/z 225 while cleavage at alpha to the epoxy group gave rise to a fragment ion at m/z 279 This could also represent the acyl group of vernolic acid. The fragment at m/z 321 was due to the loss of -$CH_2CH_2CH_2CH_2CH_3$ group while that at 363 was due to the loss of -CH_2CH_3 group.

Chemical ionization (CI) spectrometry gave rise to a similar fragmentation pattern where the CH_4 induced CI spectrum shows the quasimolecular ion (MH, m/z 393.306) as the base peak

(100%). The peak at m/z 421.346 was due to the $MC_2H_5^-$ ion. The NMR data related to different

functionalities are given in Table 3.

Table 3 ^{1}H- NMR and ^{13}C- NMR Data (δ in ppm) for N-2-(1-pyrrolidino)ethylvernolamide

	δ (in ppm)		δ (in ppm)
NH	6.30	—CH—CH— (with O epoxide)	56.54 - 57.21
—CH=CH—	5.42 - 5.65	—N(CH₂—)(CH₂—)	53.81 - 54.71
—CH₂N—	3.39 - 3.48		
—CH—CH— (with O epoxide)	2.97 - 3.02	—CH=CH—	123.86 - 132.57
—N(CH₂—)(CH₂—)	2.58 - 2.71	C=O	173.25
—CH₂—C— (with O)	2.09 - 2.42		
—CH₂—C=C<	2.09 - 2.42		
—(CH₂)ₙ—	1.32 - 1.89		
—CH₃	0.90		

3.2.2 Reaction of vernonia oil with 4-(3-aminopropyl)morpholine

The reaction resulted in the formation of an amide that still contained the epoxy group in the

molecule as confirmed by IR with absorptions at 825 cm^{-1} and 840 cm^{-1}. Sharp absorptions at 1640

cm^{-1} and 1550 cm^{-1} corresponding to amide I (C=O) and amide II (NH) bands, respectively, were also observed. A very sharp peak at 3300 cm^{-1} due to NH stretching indicated it was a secondary amide.

The peak at m/z 424 in the EI MS represents the M+2 ion while the base peak at m/z 100 is due to the cleavage of the C-C bonds next to the nitrogen in the ring. Cleavage between C-11 and C-12 (allylic cleavage) gave a fragment ion at m/z 309. The ion at m/z 351 was attributed to cleavage alpha to the epoxy group (C13-C14) while a McLafferty rearrangement gave the ion at m/z 186. Alpha cleavage of the group attached to the amide nitrogen gave rise to a fragment ion at m/z 114 and β- cleavage of C-C bond to the carbonyl group gave rise to a fragment ion at m/z 199.

The CH$_4$ induced CI spectrum shows the quasimolecular ion ion (MH+, m/z 423.416) as the base peak (100%) and a peak at m/z 451.457 was due to MC$_2$H$_5^+$ ion. Other major peaks were observed at m/z 100.088 (15%), 279.272 (6%) and 351.319 (10%).
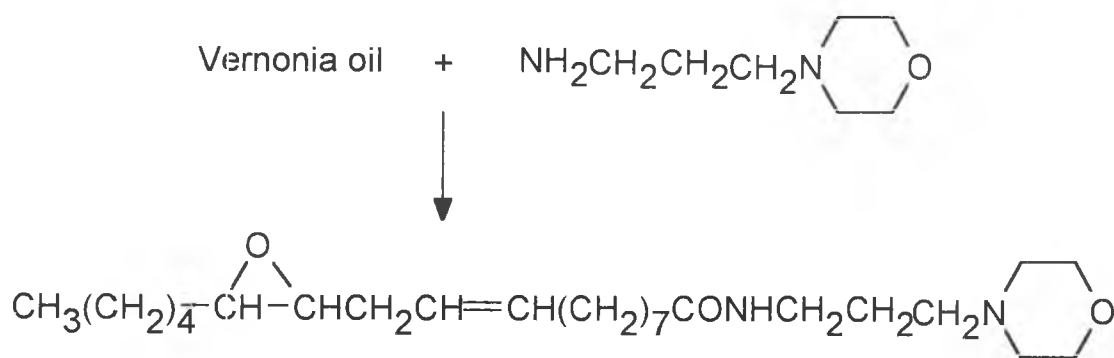
Scheme 3: Synthetic scheme for N-3-(4-morpholino)propylvernolamide

The NMR spectra was consistent with the structure. Protons on the terminal methyl groups gave a peak at 0.9 ppm, methylene group protons gave signals between 1.30 and 1.80 ppm except for the allylic methylenes (2.20 ppm and 2.45 ppm), the methylene alpha to the carbonyl functionality (2.05 ppm) and the methylene adjacent to the NH group (3.35 ppm). The epoxy hydrogens produced signals at 2.9-3.0 ppm, while the olefinic hydrogens gave complex signals at

5.4 and 5.6 ppm. The broad singlet at 6.85 ppm was due to the NH proton. The -CH$_2$O protons in the morpholine ring show an absorption at 3.8 ppm, while the methylenes adjacent the nitrogen in the ring absorb at 2.45 ppm.

^{13}C-NMR of the amide also confirmed the presence of the epoxy carbons at 56.53 and 57.87 ppm. Olefinic carbons gave signals at 123.91 and 132.53 ppm, while the carbonyl carbon had a peak at 172.89 ppm. The peak at 67.05 ppm was attributed to the methylene carbons adjacent to the oxygen in the morpholine ring, while the methylenic carbons adjacent to the nitrogen in the ring absorb at 53.72 ppm.

3.2.3 Formation of N-3-[1-(2-oxopyrrolidino)]propylvernolamide

Reaction of vernonia oil with 1-(3-aminopropyl) -2-pyrrolidinone also resulted in the formation of an amide which was identified by IR, EI MS, CI MS and NMR.
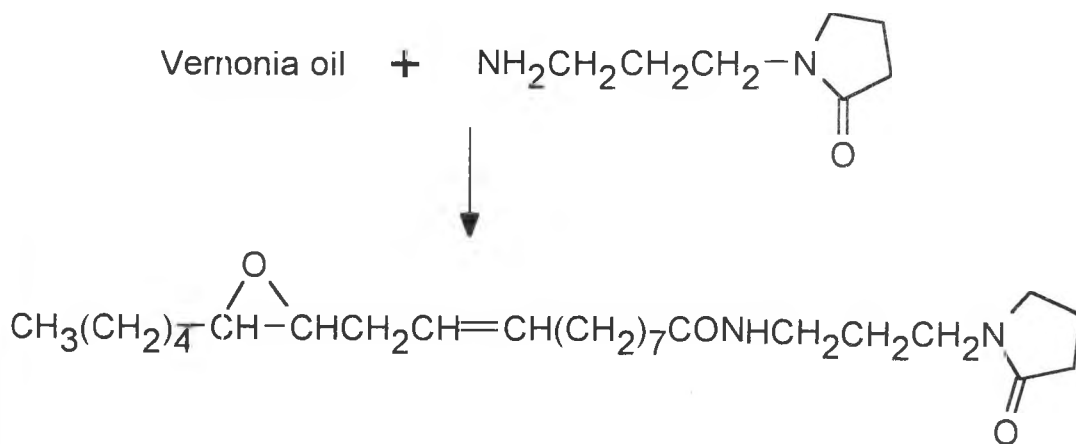
Scheme 4: Synthetic scheme for N-3-[1-(2-oxopyrrolidino)]propylvernolamide

The absorption at 1680 cm^{-1} in the IR spectrum was due to C= 0 strecthing in the pyrrolidinone ring, while the C = 0 stretching due to the amide bond formed was observed at 1645 cm^{-1}.
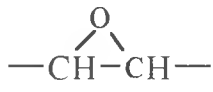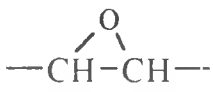
Mass-spectral data indicated fragment ions that were similar to those previously described for N-2-(1-pyrrolidino)ethylvernolamide and N-3-(4-morpholino)propylvernolamide. The molecular

31

The absorption at 1680 cm^{-1} in the IR spectrum was due to C= 0 strecthing in the pyrrolidinone ring, while the C = 0 stretching due to the amide bond formed was observed at 1645 cm^{-1}.

Mass-spectral data indicated fragment ions that were similar to those previously described for N-2-(1-pyrrolidino)ethylvernolamide and N-3-(4-morpholino)propylvernolamide. The molecular ion [M+.] was observed at m/z 420. The base peak at m/z 126 is due to the fragment ion from the cleavage at the alpha carbon to the NH group while a McLafferty rearrangement gave rise to a peak at m/z 184. Allylic cleavage of bonds between C-11 and C-12 gave a fragment at m/z 307 and the peak at m/z 143, could be due to a quarternary ammonium ion. The ion at m/z 349 was attributed to cleavage alpha to the epoxy group (C13 - C14) while the fragment at m/z 279 could represent an ion attributable to the acyl group of vernolic acid. Fragment ions at m/z 169 and 197 could result from simple cleavages alpha and gamma to the carbonyl group respectively.

The CH$_4$ induced CI spectrum shows the quasimolecular ion (MH$^+$, m/z 421.268) as the base peak (100%) and the peak at m/z 449.308 was due to the MC$_2$H$_5$$^+$ ion. Other major significant peaks were observed at m/z 126.078 (15%) and 321.201 (6%).

Both the ^{1}H-NMR and ^{13}C-NMR spectra were consistent with the structure (table 4, page 33). The carbonyl carbons are further downfield, and were very weak. The spectrum supports the structure by having the right number of signals for the carbon atoms present, and in all appropriate regions.

Table 4 ^{1}H- and ^{13}C-NMR Data (δ in ppm) of N-3-[1-(2-oxopyrrolidino)]propyl-

vernolamide

	δ (in ppm)		δ (in ppm)
NH	6.71		
—CH=CH—	5.30 - 5.58	—CH–CH— (O bridge)	56.60 - 57.25
—CH$_2$N—	3.16 - 3.43	—CH=CH—	123.89 - 132.68
—CH–CH— (O bridge)	2.90 - 2.92		
—CH$_2$–C— (C=O)	2.02 - 2.42		
—CH$_2$–C=C—	2.02 - 2.42		
—(CH$_2$)n—	1.30 - 1.70		
CH$_3$—	0.91		

3.2.4 Reaction of vernonia oil with 1-(3-aminopropyl)imidazole

Reaction of vernonia oil with 1-(3-aminopropyl)imidazole in dimethylformamide (the reaction did not proceed without the solvent) resulted in the formation of a new compound, N-3-(1-imidazole)propylvernolamide. The solvent dilutes the reactants, therefore, the reaction took a longer time (48 hours) to go to completion. This was attributed to the lipophilic nature of the amine and hence does not react readily with the triglycerides due to nsolubility without the solvent. It was observed that 1-(3-aminopropyl)imidazole and vernonia oil were immiscible at room temperature and even at the temperature of the reaction (50°C) while 1-(2-aminoethyl)pyrrolidine, 4-(3-

3.3 Ring opening reactions of vernolamides

3.3.1 Reaction of N-2-(1-pyrrolidino)ethylvernolamide with dilute acid

The equation below shows the reaction of N-2-(1-pyrrolidino)ethylvernolamide with dilute acid.

$$CH_3(CH_2)_4-CH-CHCH_2CH=CH(CH_2)_7CONHCH_2CH_2N \quad + \quad H_2O$$

(epoxide O bridging the CH–CH)

$$\downarrow H^+$$

$$CH_3(CH_2)_4-CH-CHCH_2CH=CH(CH_2)_7CONHCH_2CH_2N$$
(with OH OH on the CH–CH)

12,13-dihydroxy-N-2-(1-pyrrolidino)ethyl-9-octadecenamide

In the infrared spectrum of the compound, a strong, broad band in the 3200 - 3600 cm^{-1} region was due to O-H stretching. The $C = O$ stretching band was still present but lowered to 1630 cm^{-1} while another strong, broad band, due to C-O stretching appeared at 1150 cm^{-1}. The bands at 825, and 840 cm^{-1} were not observed in the spectrum indicating that the epoxy group had undergone ring-opening.

EI MS gave the following significant peaks:-

43 (12.3), 84.3 (100), 169.2 (3.8), 279.5 (10), 309 (30.8) and 374.3 (11.53).

The base peak at m/z 84 was due to the beta cleavage to the NH group. Allylic cleavage (m/z 279.5) established the double bond at C(9) and C(10) while cleavage between the hydroxy groups gave the signal at m/z 309. The peak at m/z 374.3 was attributed to the loss of two water molecules.

gave the signal at m/z 309. The peak at m/z 374.3 was attributed to the loss of two water molecules

3.3.2 Reaction of N-2-(1-pyrrolidino)ethylvernolamide with Acetic Acid.

Reaction of acetic acid with the vernolamide resulted in the formation of a yellow oil. In the IR spectrum of the product the absorption due to the epoxy group (825, 840 cm^{-1}) had disappeared while a new absorption band at 3200-3600 cm^{-1} indicating that the epoxy group had opened up, giving a product with a hydroxy group. The amide C = O stretching band was still present at 1630cm^{-1} and C=O bond of the ester functionality appears at 1640 cm^{-1} probably due to intramolecular hydrogen bonding.

The EI MS of the derivative gave significant peaks at m/z 42.8 (11.5)), 84 (100) 239.3 (5.4), 279.3 (6.2), 309 (6.9), 350.4 (8.5) 374.4 (8.5) and 406.3 (13.8).

Where

$$R_1 = \text{(pyrrolidine ring)} NCH_2CH_2NHCO(CH_2)_7-CH=CH-CH_2-$$
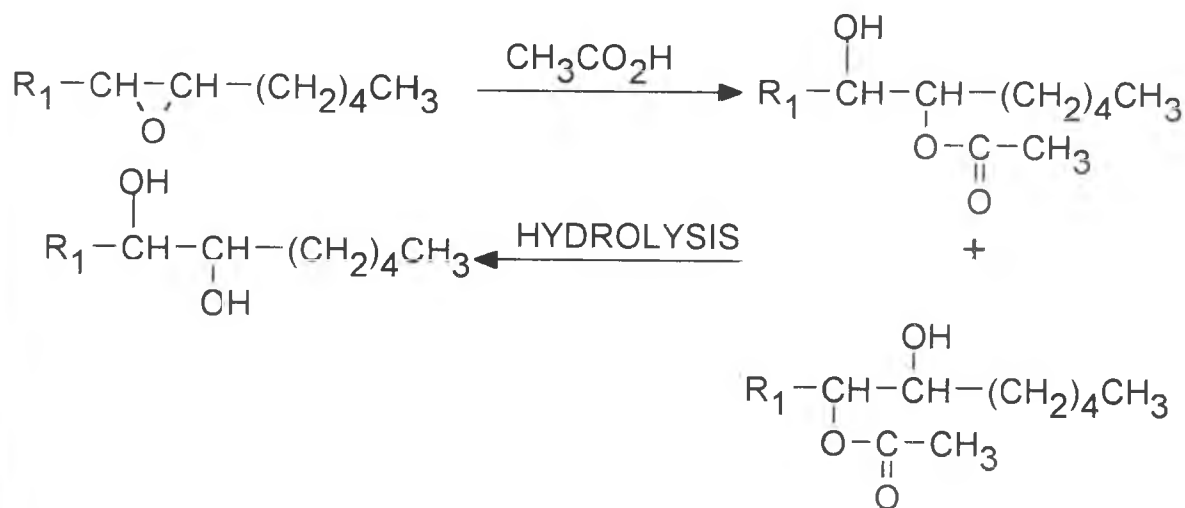
Scheme 5: Reaction scheme for the synthesis of the hydroxy ester

The method depends on the strength of the acid RCO_2H (i.e. on its ability to protonate the oxygen of the oxiran). Formic and trifluoroacetic acids are sufficiently strong to effect the ring opening, but are avoided because they react with the double bond. The hydroxy ester and dihydroxy amides were confirmed by the hydroxamic acid test and acetyl chloride.

3.4   The Biological activity of the epoxy amides

3.4.1   Antibacterial activity

The antibacterial activities of N-2-(1-pyrrolidino)ethylvernolamide, N-3-[1-(2-oxopyrrolidino)]propylvernolamide and N-3-(4-morpholino)propylvernolamide against *Staphylococcus aureus* (gram-positive), *Escherichia coli* (gram-negative) and *Bacillus subtilis* are given in table 5. The screening of activity was carried out using the amines and their derivatives in chloroform. The activity of the amine derivatives was greater against the gram-negative bacteria than the gram-positive bacteria as shown by the inhibition zones. The gram-positive bacteria were inhibited to a small extent by the vernolamides. The absence of activity by the starting amines highlighted the enhanced effect of the derivatives.

Table 5. Antibacterial Activity of Epoxy Amides by the disc method

Compound	Concentration (μg)	*Escherichia coli* diameter (mm)	*Staphylococcus aureus* diameter (mm)	Bacillus *subtlis* diameter (mm)
N-2-(1-pyrrolidino)ethyl-vernolamide	100	20	-	10
	50	20	-	8
	25	8	-	-
N-3-(4-morpholino)propyl-vernolamide	100	25	-	8
	50	25	-	6
	25	-	-	-
N-3-[1-(2-oxopyrrolidino)]propyl-vernolamide	100	30	10	-
	50	25	10	-
	25	-	-	-

3.4.2 Antifungal activity

The antifungal activities of the derivatives against *Trichophyton mentagrophyte, Microsporum gypseum,Candida albicans* and *Saccharomyces cerevisiae* are given in table 6. The activity of N-2-(1-pyrrolidino)ethylvernolamide was more pronounced against *Trichophyton mentagrophyte* than against the other fungi. All the vernolamide derivatives showed inhibition against *Saccharomyces cerevisiae,Trichophyton mentagrophyte* and *Microsporum gypseum.* The presence of additional bonds (pyrrolidine and morpholine rings) in the epoxy acid resulted in enhanced inhibition as compared to vernonia oil.

The amines, (1-(2-aminoethyl)pyrrolidine, 1-(3-aminopropyl)- 2-pyrrolidinone and vernonia oil did not show any activity against the microorganisms tested (both bacteria and fungi) except 4-(3-aminopropyl)morpholine which was active against *Eschericha coli* at a concentration of 500 µg.

Table 6: Antifungal Activity of Epoxy Amides by the Disc method

Compound	Concentration (µg)	*Saccharomyces cerevisiae* diameter (mm)	*Trichophyton mentagrophyte* diameter (mm)	*Microsporum gypseum* diameter (mm)	*Candida albicans* diameter (mm)
N-(2-pyrrolidino)ethyl-vernolamide	100 50 25	10 10 8	20 20 8	10 10 8	- - -
N-3-(4-morpholino)propyl-vernolamide	100 50 25	8 8 -	10 10 8	10 10 -	- - -
N-3-[1-(2-oxopyrrolidino)]propy-vernolamide	100 50 25	12 10 -	8 - -	8 - -	- - -

3.5 CONCLUSION AND RECOMMENDATIONS

The study has shown that under appropriate conditions, vernonia oil reacts with 1-(2-aminoethyl)pyrrolidine, 1-(3-aminoprpropyl)-2-pyrrolidinone, 4-(3-aminopropyl)morpholine and 1-(3-aminopropyl)imidazole to form their respective vernolamides. It was observed that the best yield was obtained from the reaction between 1-(2-aminoethyl)pyrrolidine and vernonia oil. The synthesized vernolamides contain tertiary amino groups.

The chemical form of the amine moiety has an effect on amidation as reflected in the yields of the products. The pyrrolidine, morpholine , pyrrolidinone and imidazole rings do not affect the reactivity of the amines to a great extent because they are not attached directly to the -NH_2 group. However, solubility of the reactants influences the rate of a reaction. There was no product obtained from the reaction of vernonia oil with 1-(3-aminopropyl)imidazole in the absence a solvent. But on dissolving both the oil and the amine in dimethylformamide, a product was obtained.

The opening of the epoxy group demonstrates the possibility of making new derivatives with extended chains. The reaction of the epoxy amides with mild acids, such as acetic acid and acrylic acid, may lead to derivatization of polymers. The synthesized amide derivatives have the secondary and tertiary nitrogens separated either by two or three methylene groups. These derivatives have lone pairs of electrons on the nitrogens, therefore the compounds can function as polymeric resins for removing hydrogen ions in solution by forming quaternary ammonium ions.

This research has shown the potential of vernonia oil in synthesizing vernolamides. The synthesized vernolamides have both the double bond and epoxy functional groups hence can serve as reactive sites for the development of new synthetic products. Ring-opening reactions of the

vernolamides demonstrates the selective reactivity of the compounds under controlled conditions. This also may lead to the application of the derivatives in the chemical industry and can serve as starting compounds or key intermediates in synthetic reactions.

The vernolamides were found to be bioactive against some micro-organisms (fungi and bacteria). Thus the vernolamides could be used as bactericides and fungicides.

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APPENDIX:

SPECTRA - IR, NMR and MS

FIG. 1 – IR SPECTRUM OF VERNONIA OIL

FIG. 1 – IR SPECTRUM OF VERNONIA OIL
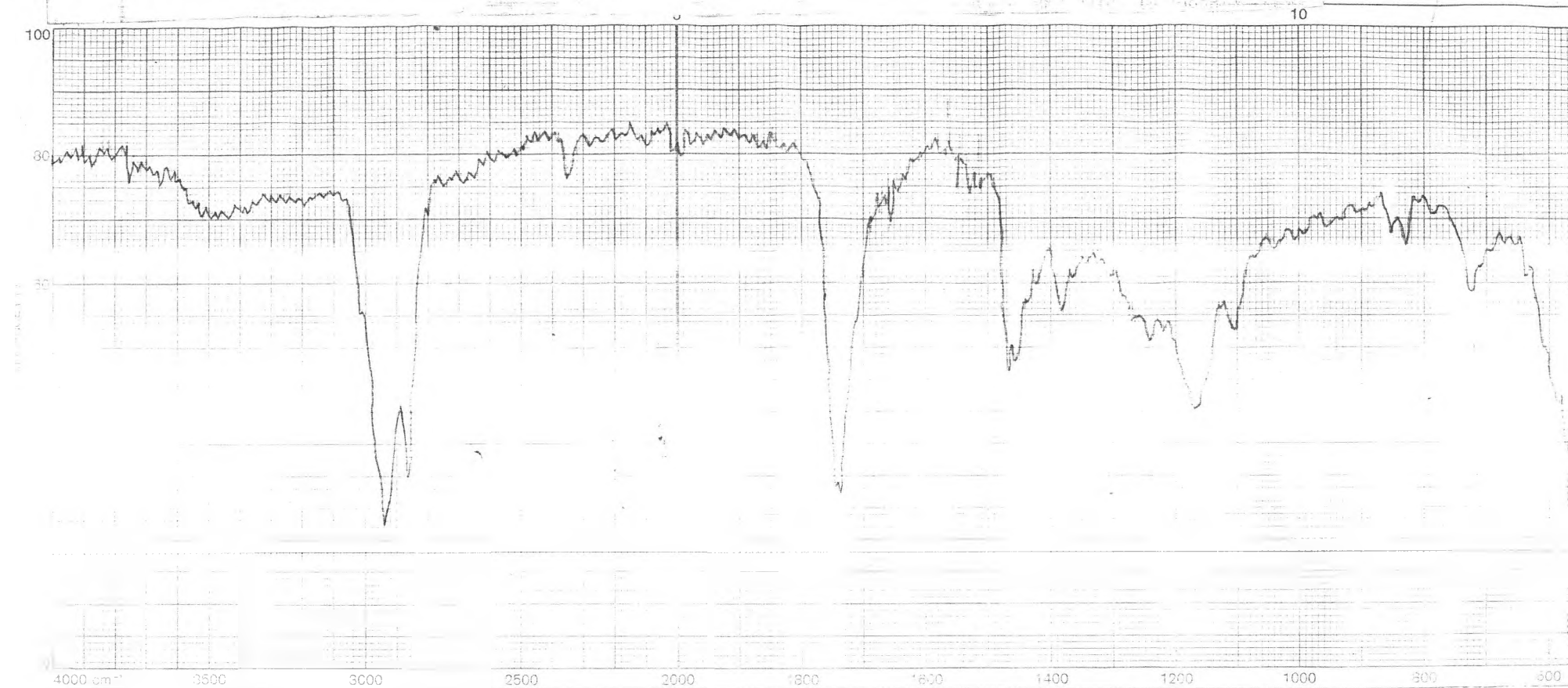
FIG. 2- IR SPECTRUM OF N-2-(1-PYRROLIDINO)ETHYLVERNOLAMIDE

46
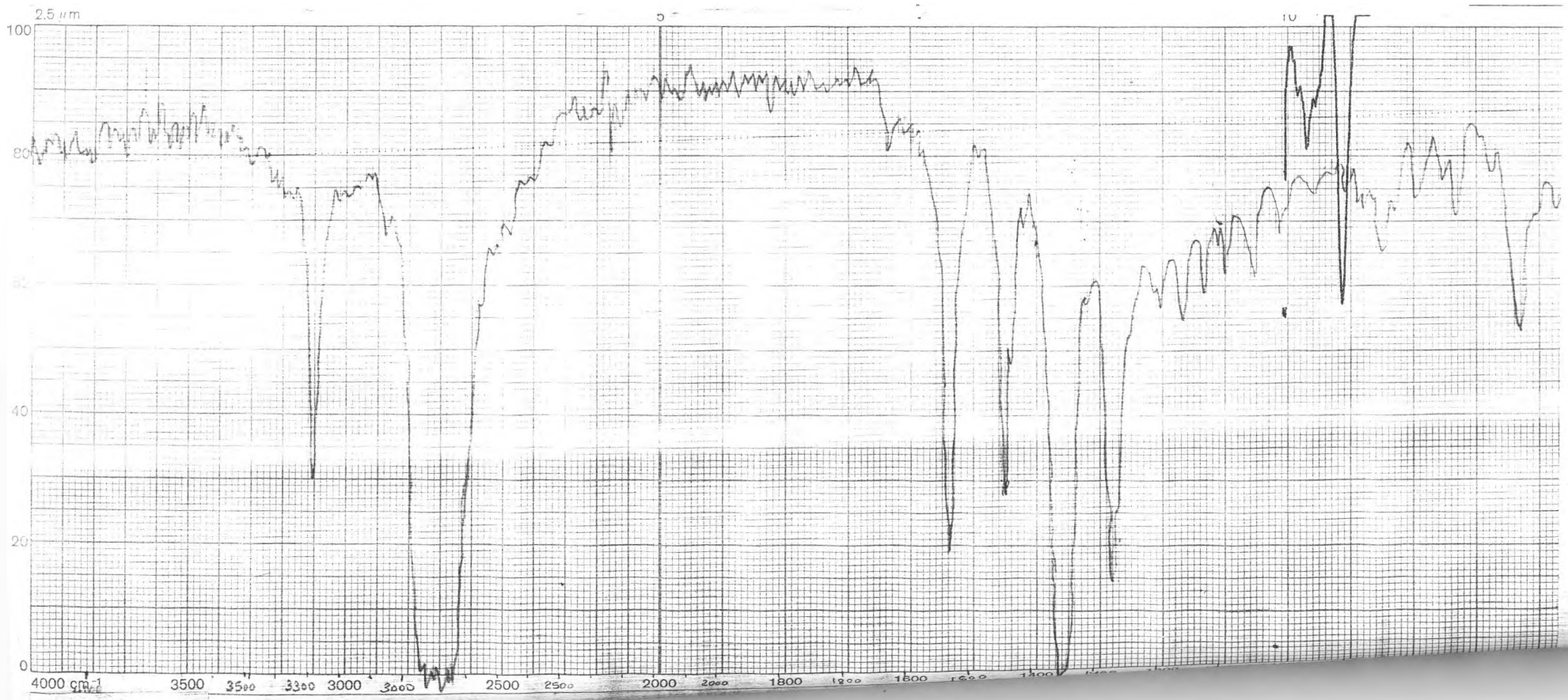
FIG.3 – EI MS OF N-2-(1-PYRROLIDINO)ETHYLVERNOLAMIDE
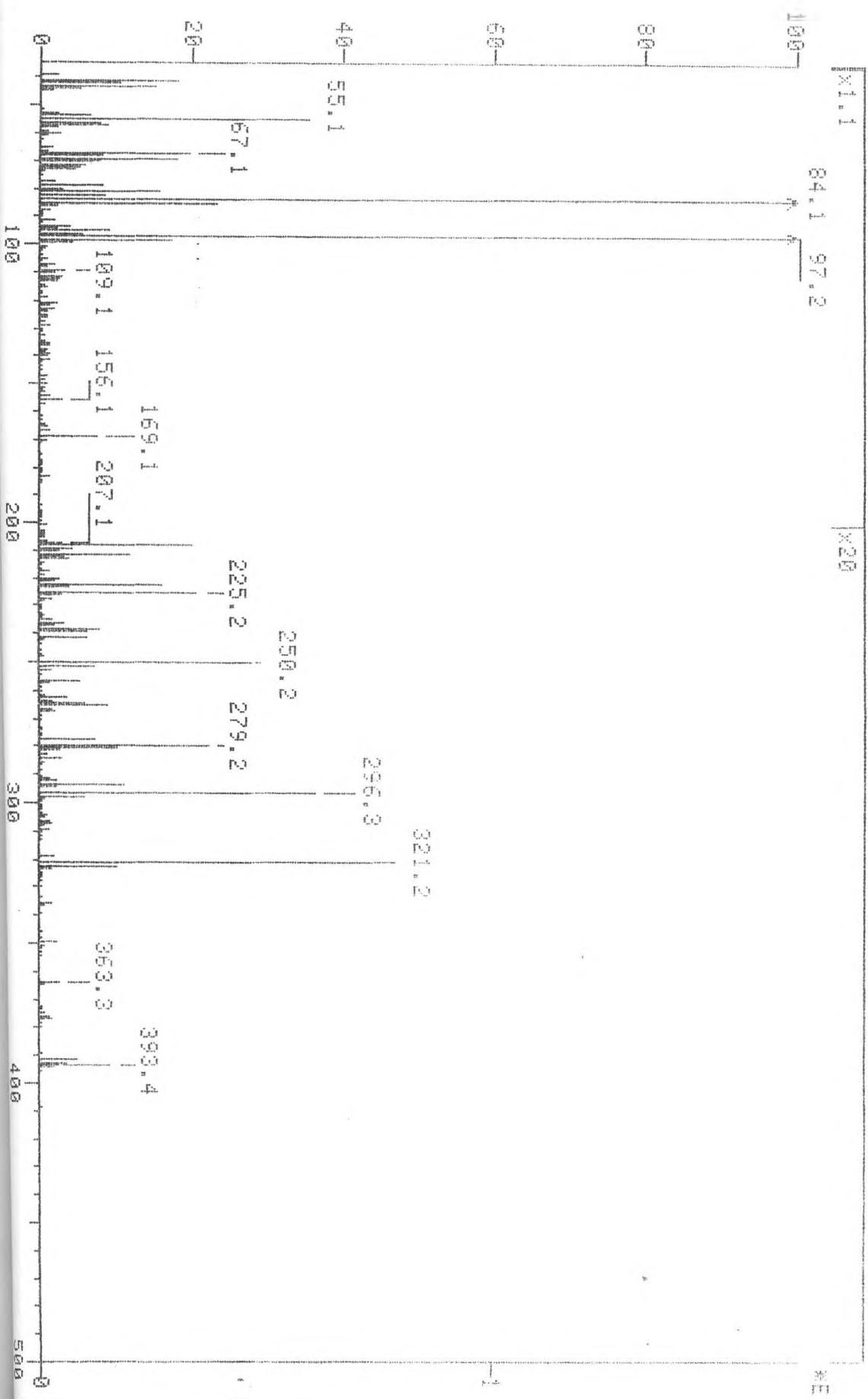
393.307
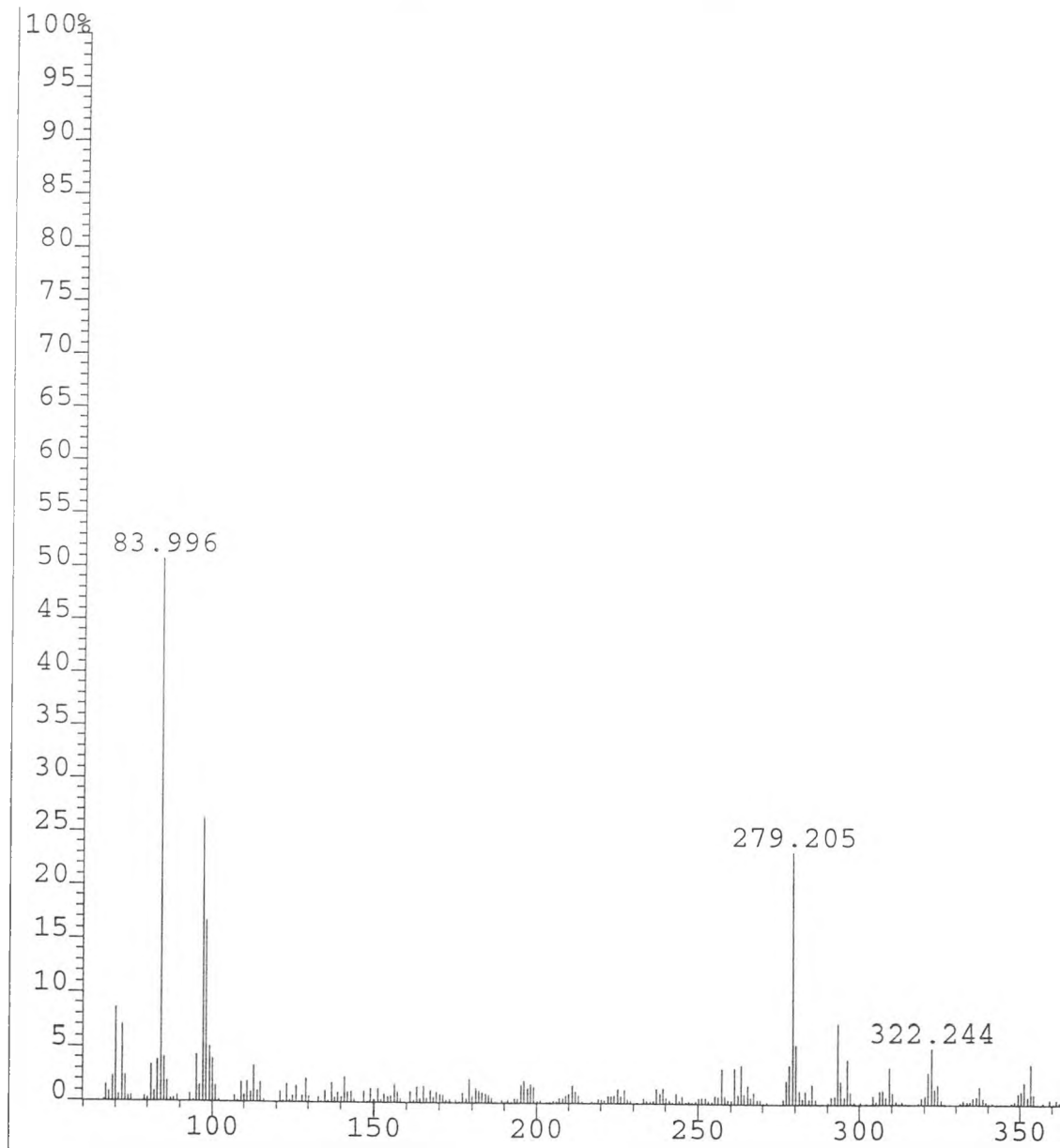
421.351

48

FIG.3— 1H NMR SPECTRUM OF (N-2-(1-PYRROLIDINO)ETHYLVERNOLAMIDE

Integral

8.0

7.5 −0.0446 — 7.2777

7.0

6.5 −0.0202 — 6.3651
— 5.5647
— 5.5379
— 5.4939
6.0 — 5.4671
— 5.4317
5.5 0.0470 — 5.4036
— 5.3878
— 5.3609
5.0

4.5

4.0 — 3.4076
— 3.3857
— 3.3625
3.5 0.0616 — 3.3393
— 3.1148
0.0415 — 2.9477
3.0 — 2.9294
0.0919 — 2.9098
— 2.6536
2.5 0.2451 — 2.6292
— 2.6060
0.0595 — 2.5707
— 2.4230
2.0 0.2533 — 2.3974
— 2.3730
0.1697 — 2.3425
— 2.3145
1.5 1.0057 — 2.2022
— 2.1741
— 2.1412
— 2.0521
1.0 0.1380 — 2.0265
— 1.8032
— 1.6202
0.5 — 1.5287
— 1.3079
— 0.9053

0.0 −0.0070 — 0.0000

(ppm)

49

FIG.6-¹³C- NMR SPECTRUM OF N-2-(1-PYRROLIDINO)ETHYLVERNOLAMIDE

ppm
— 213.003

— 173.257

— 132.571
— 123.868

— 104.894

— 77.636
— 77.001
— 76.367
— 57.211
— 56.546
— 54.754
— 53.813
— 37.851
— 36.701
— 31.710
— 29.486
— 29.242
— 29.113
— 27.717
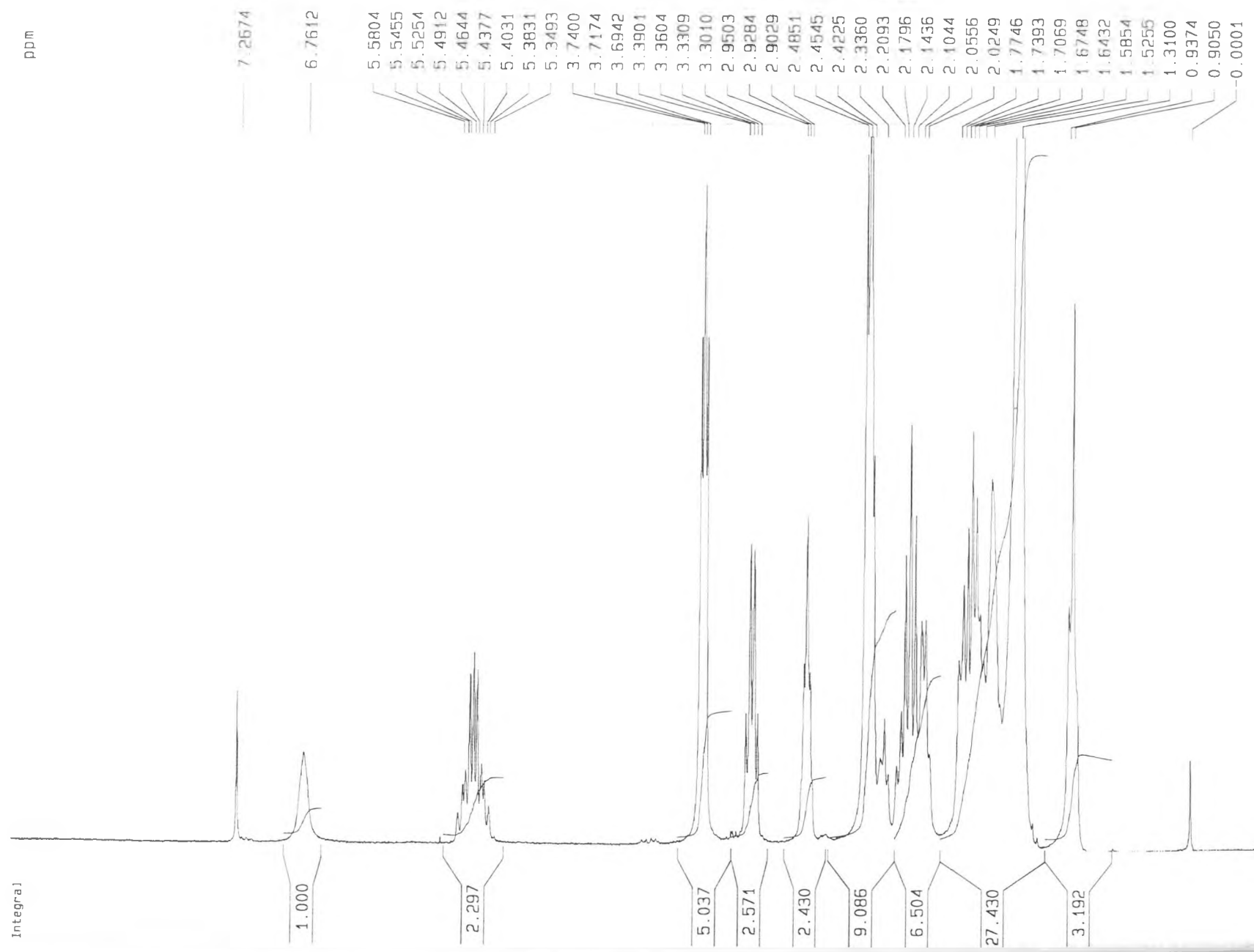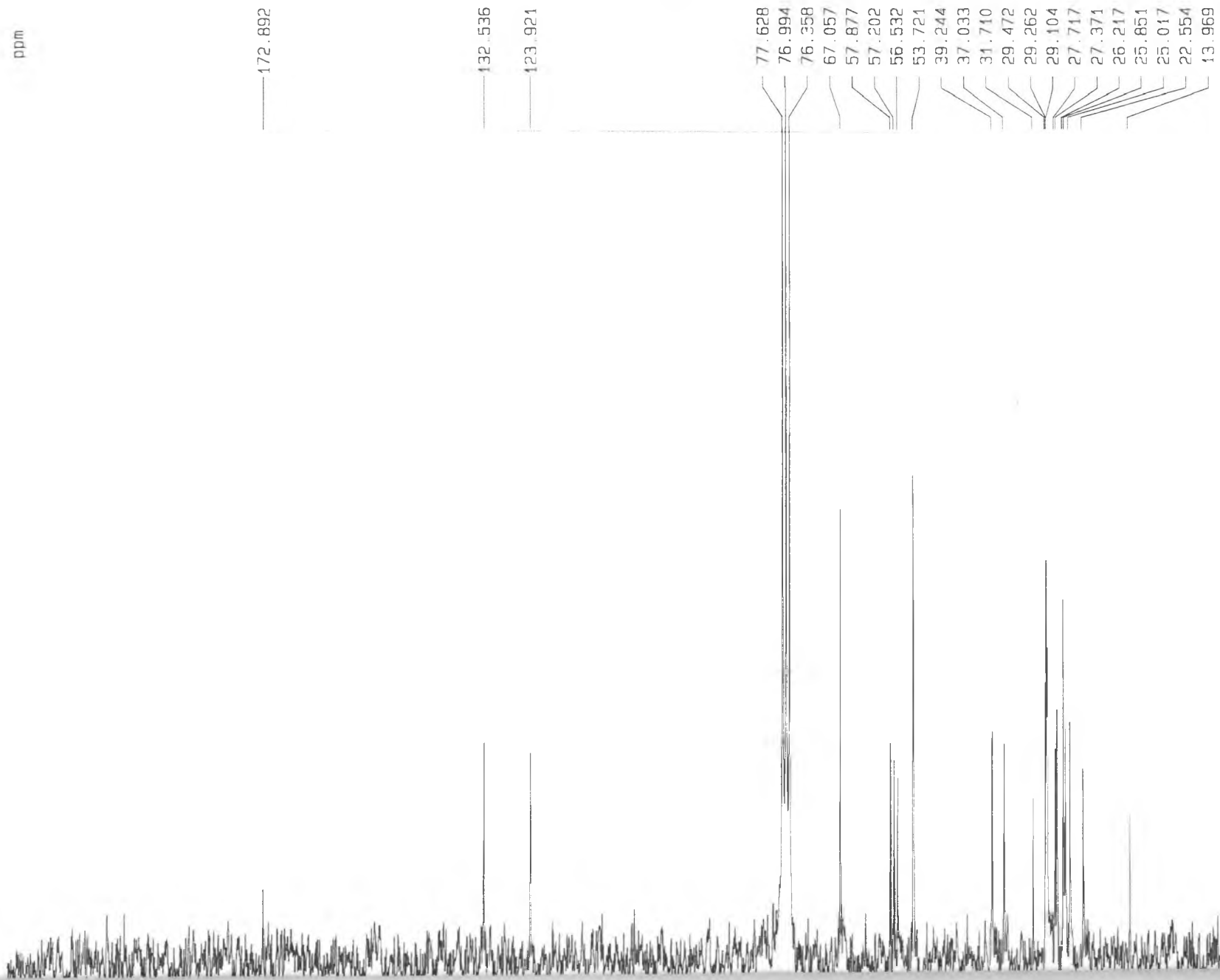— 27.383
— 26.214
— 25.720
— 24.856
— 23.420
— 22.562
— 13.977

50
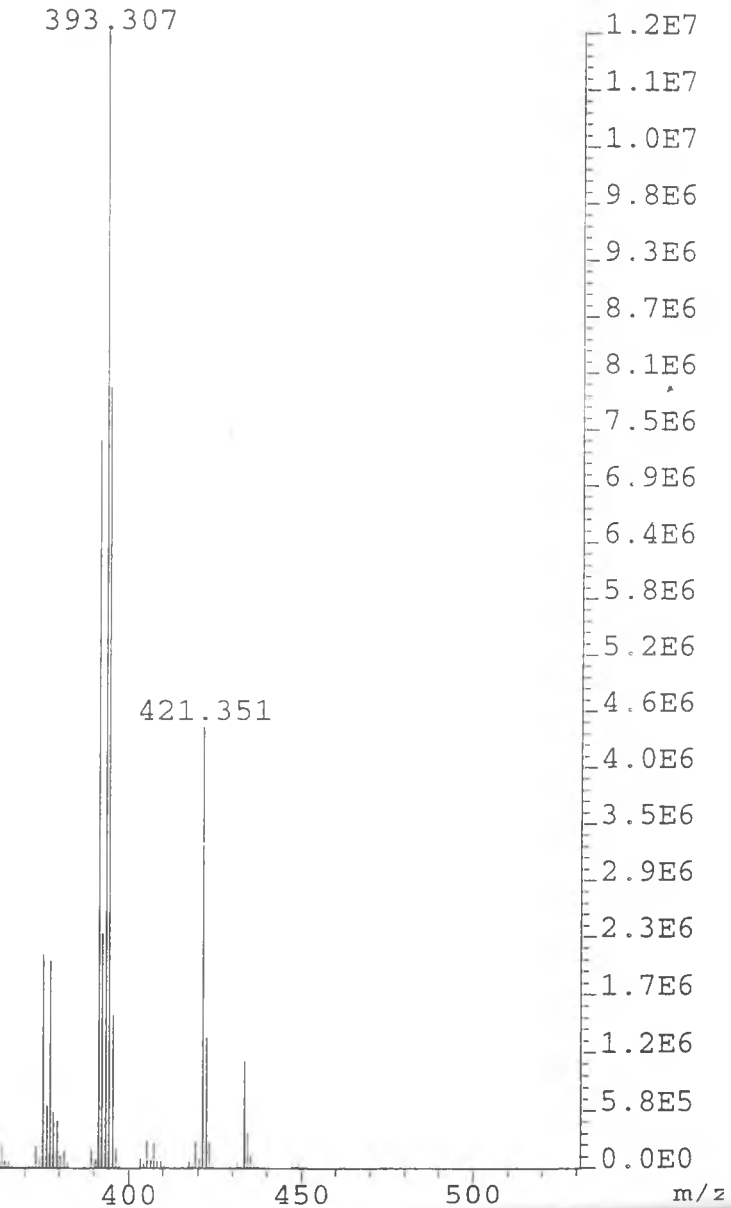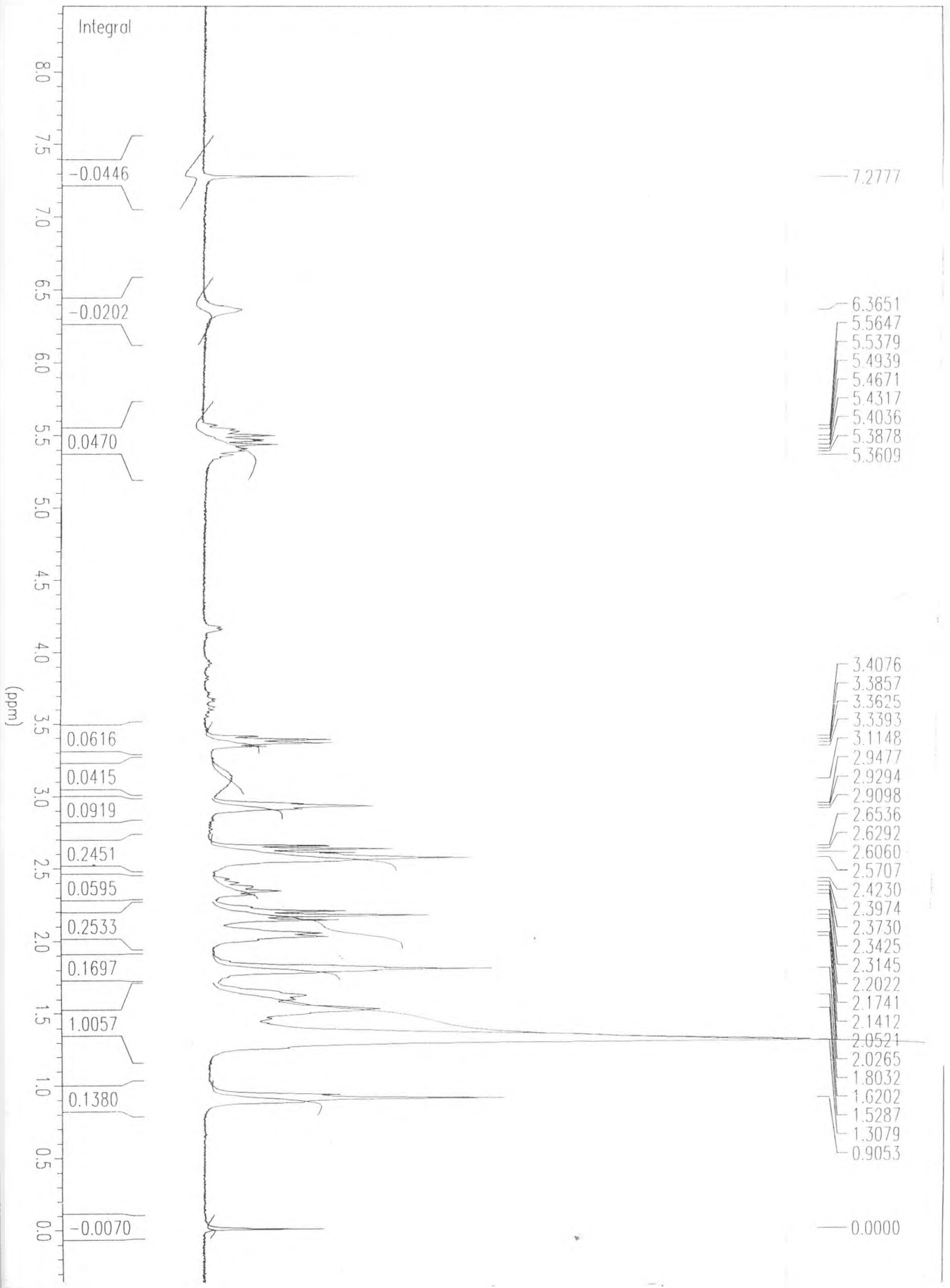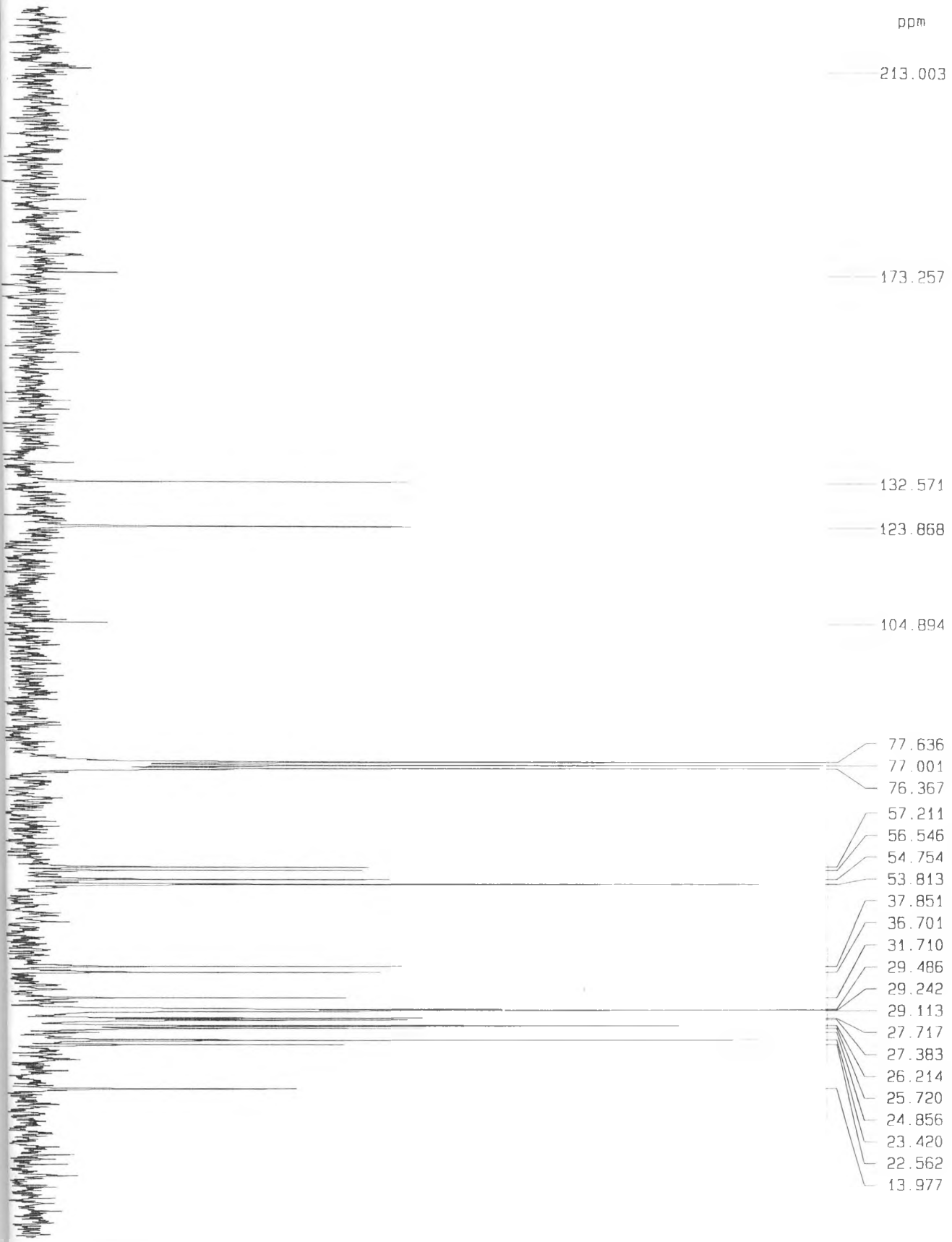
FIG. 7 —IR SPECTRUM OF N-3-(4-MORPHOLINO)PROPYLVERNOLAMIDE
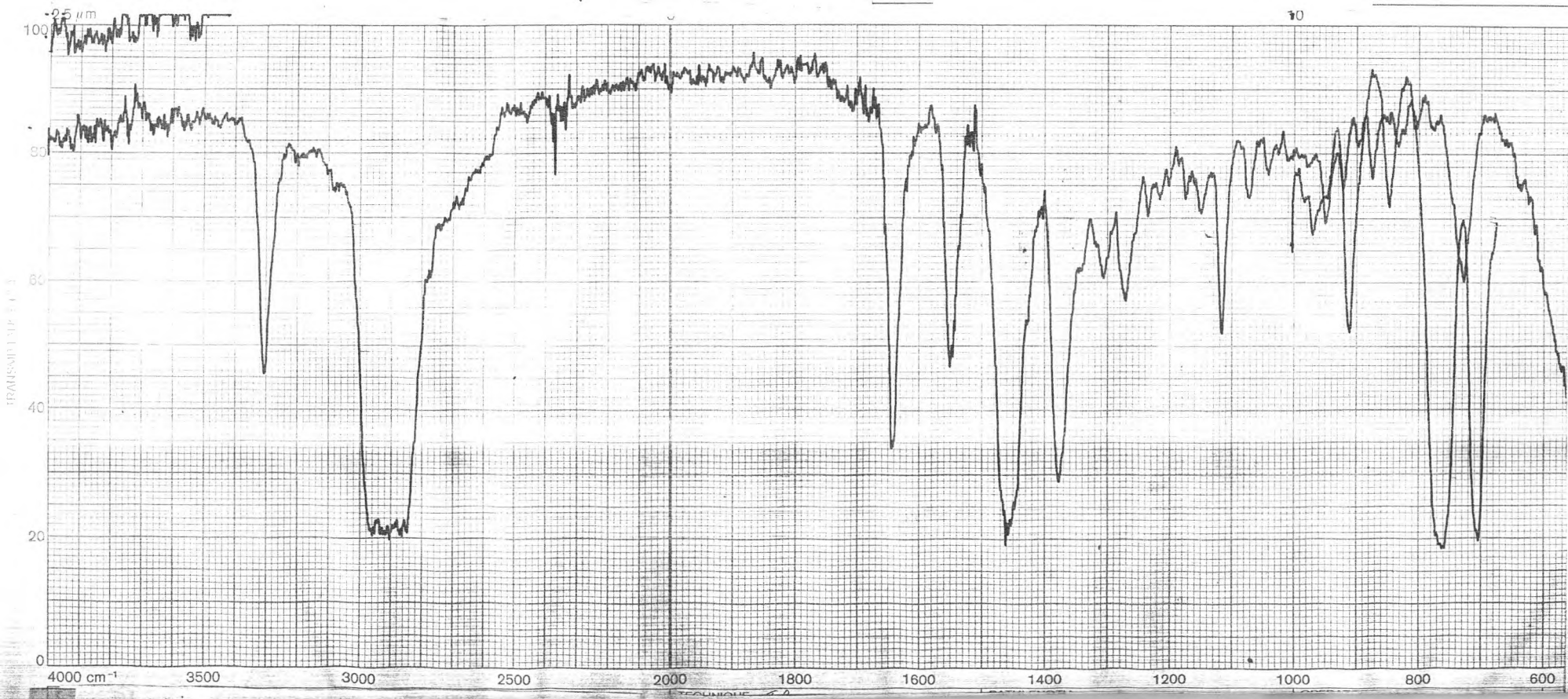
FIG.8 – EI MS OF N-3-(4-MORPHOLINO)PROPYLVERNOLAMIDE
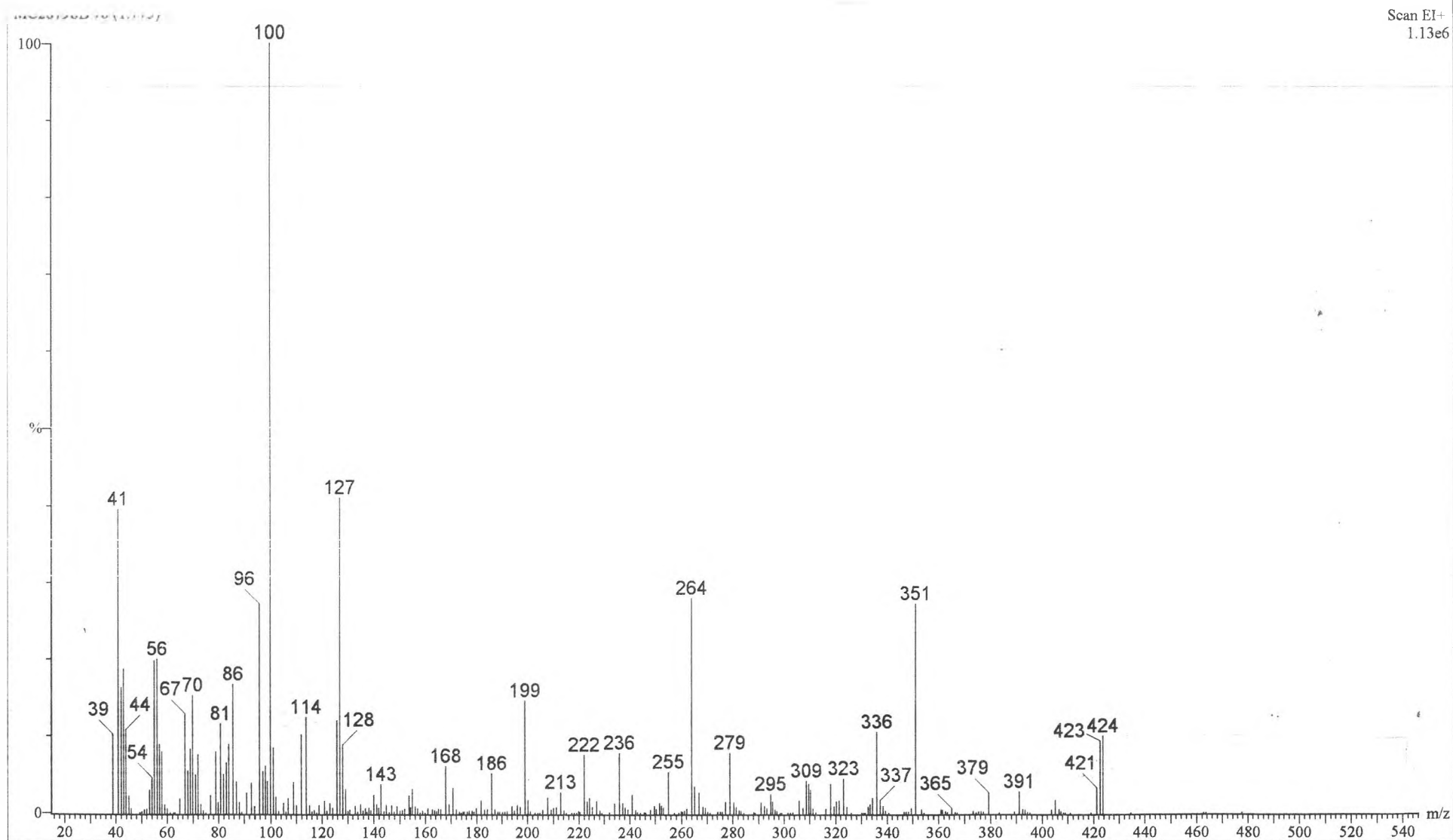
FIG. 9 – CI SPECTRUM OF N-3-(4- MORPHOLINO)PROPYLVERNOLAMIDE
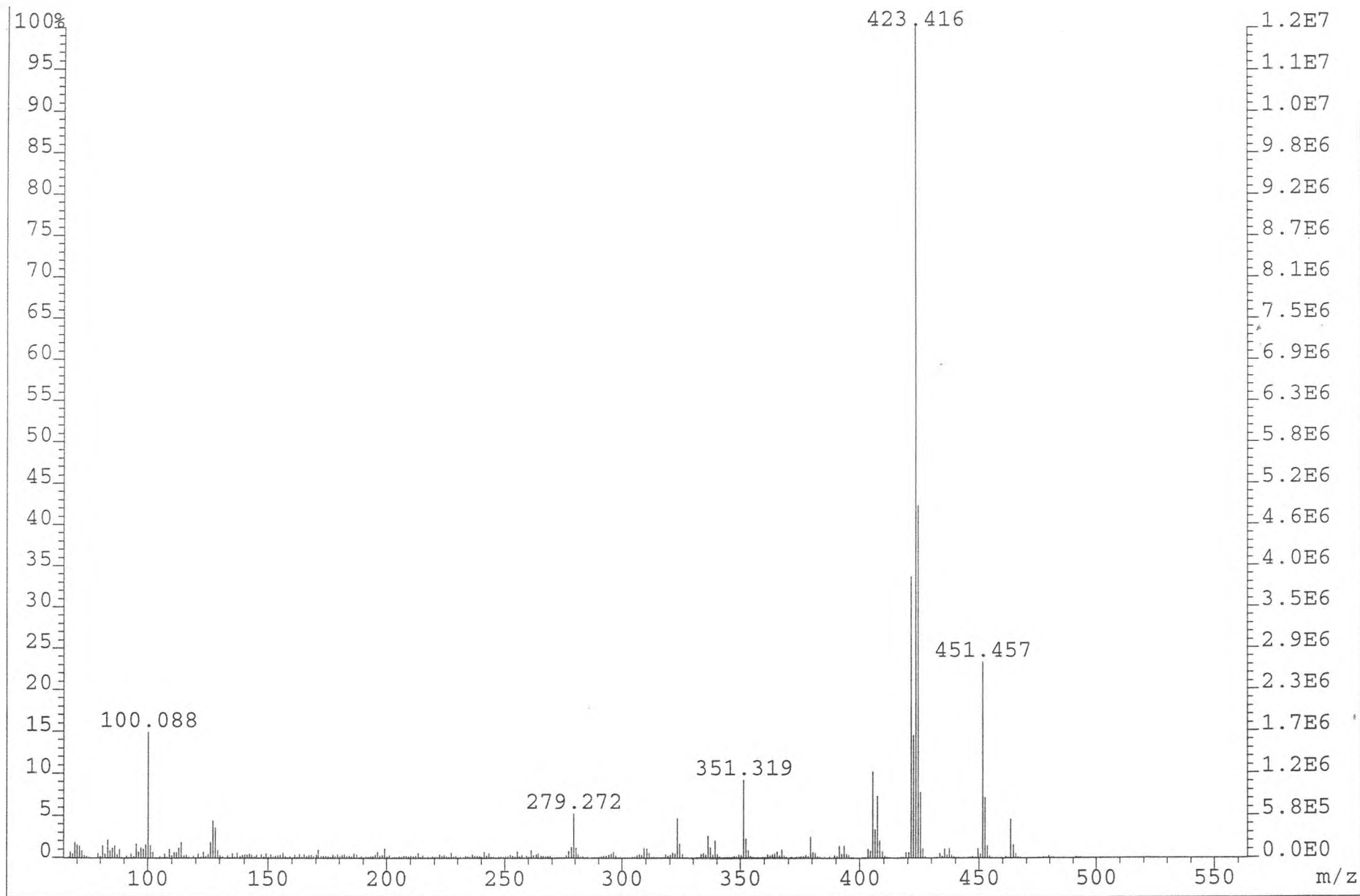
FIG.11 ¹³C- NMR SPECTRUM OF N-3- (4-MORPHOLINO)PROPYLVERNOLAMIDE

ppm

172.892
132.536
123.921
77.628
76.994
76.358
67.057
57.877
57.202
56.532
53.721
39.244
37.033
31.710
29.472
29.262
29.104
27.717
27.371
26.217
25.851
25.017
22.554
13.969
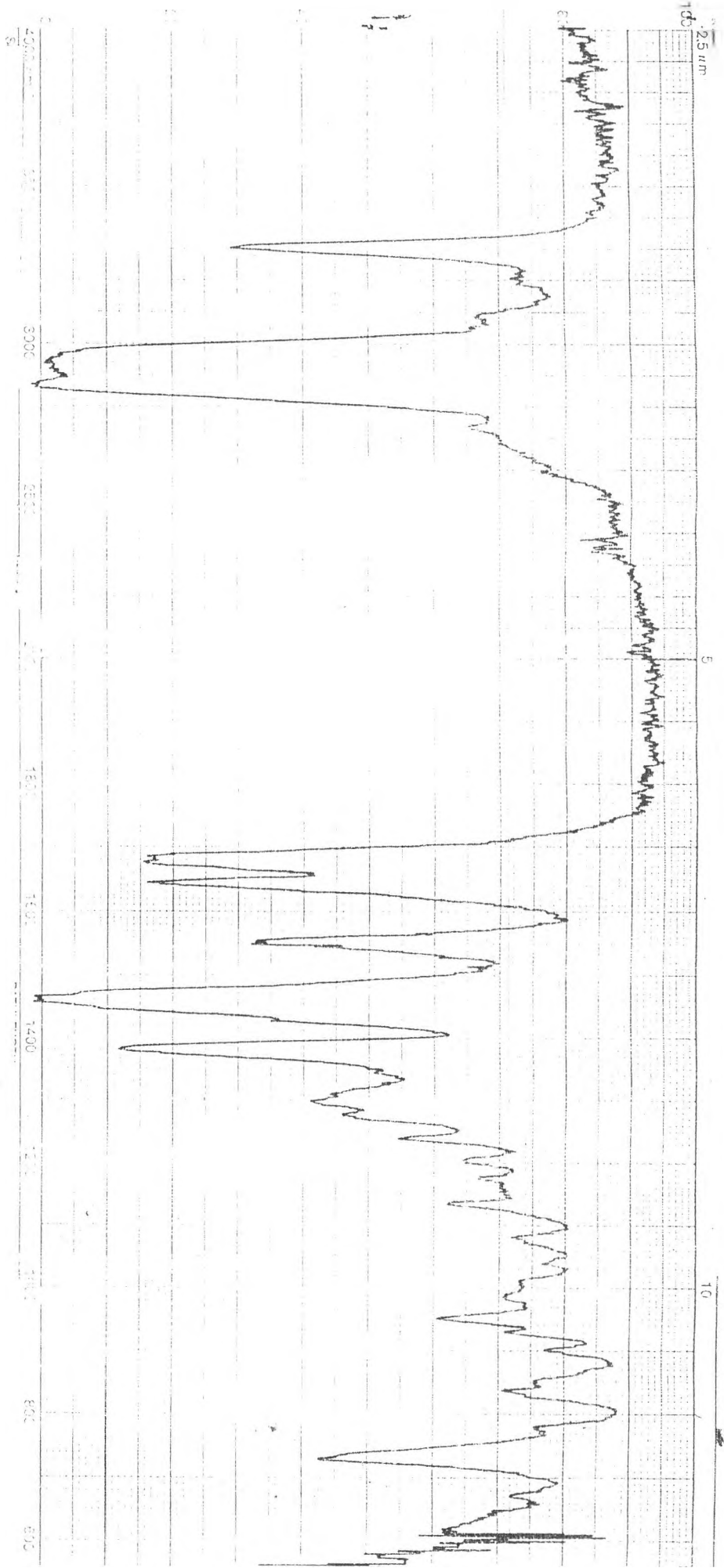
FIG.13 – EI MS OF N-3-[1-(2-OXOPYRROLIDINO)]PROPYLVERNOLAMIDE
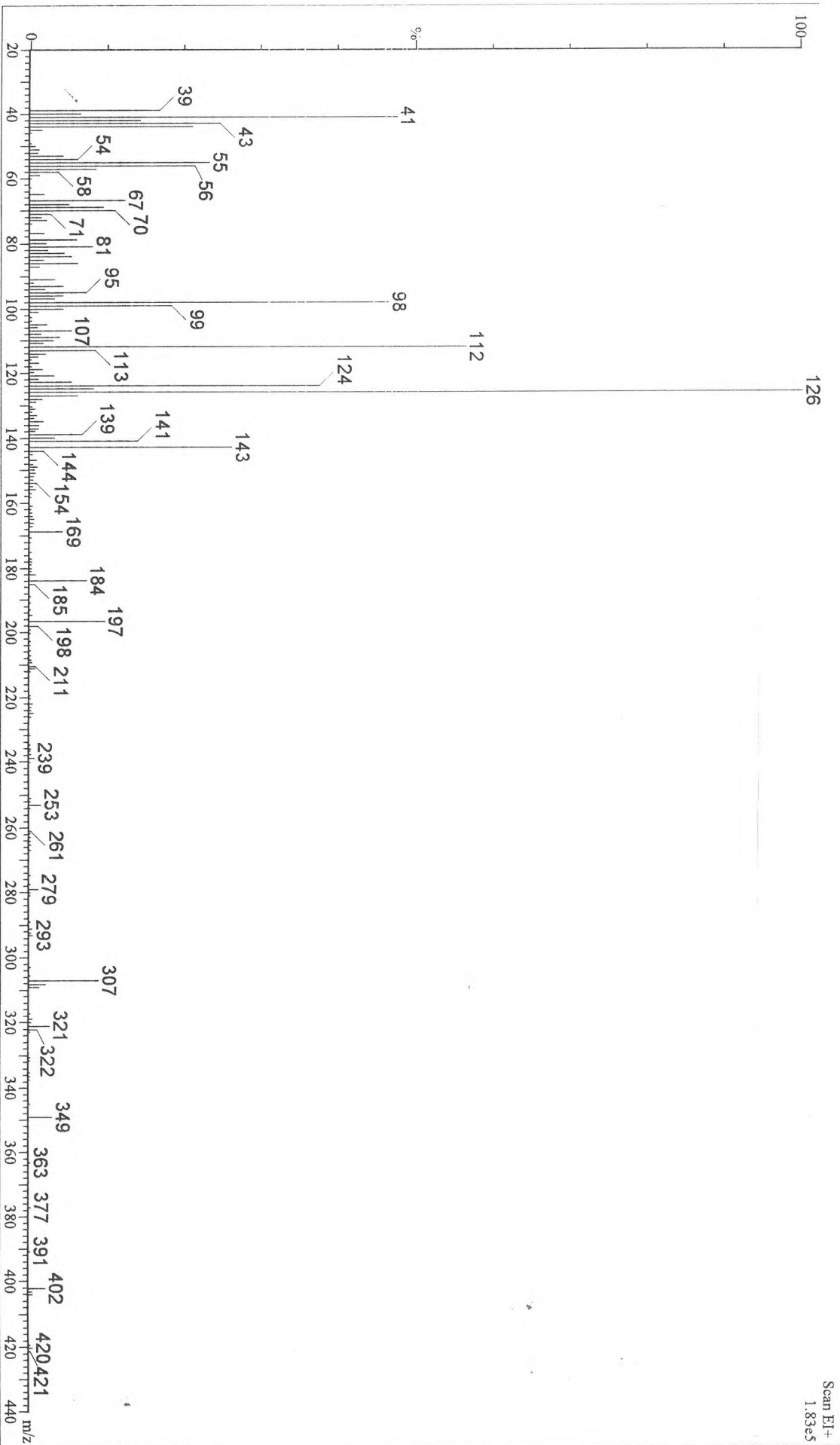
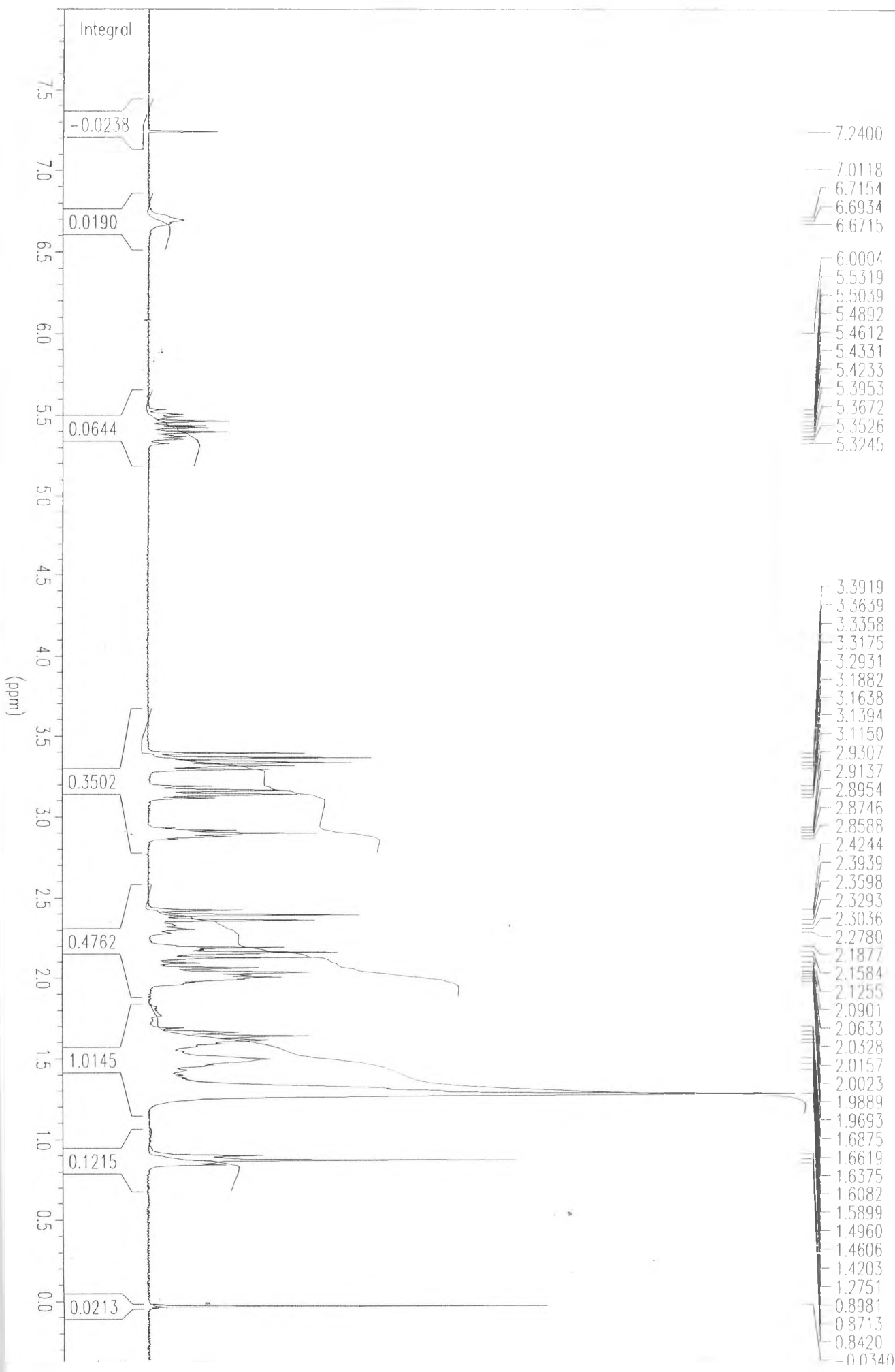FIG.14 — CI SPECTRUM OF N-3-[1-(2-OXOPYRROLIDINO)]PROPYLVERNOLAMIDE

Integral
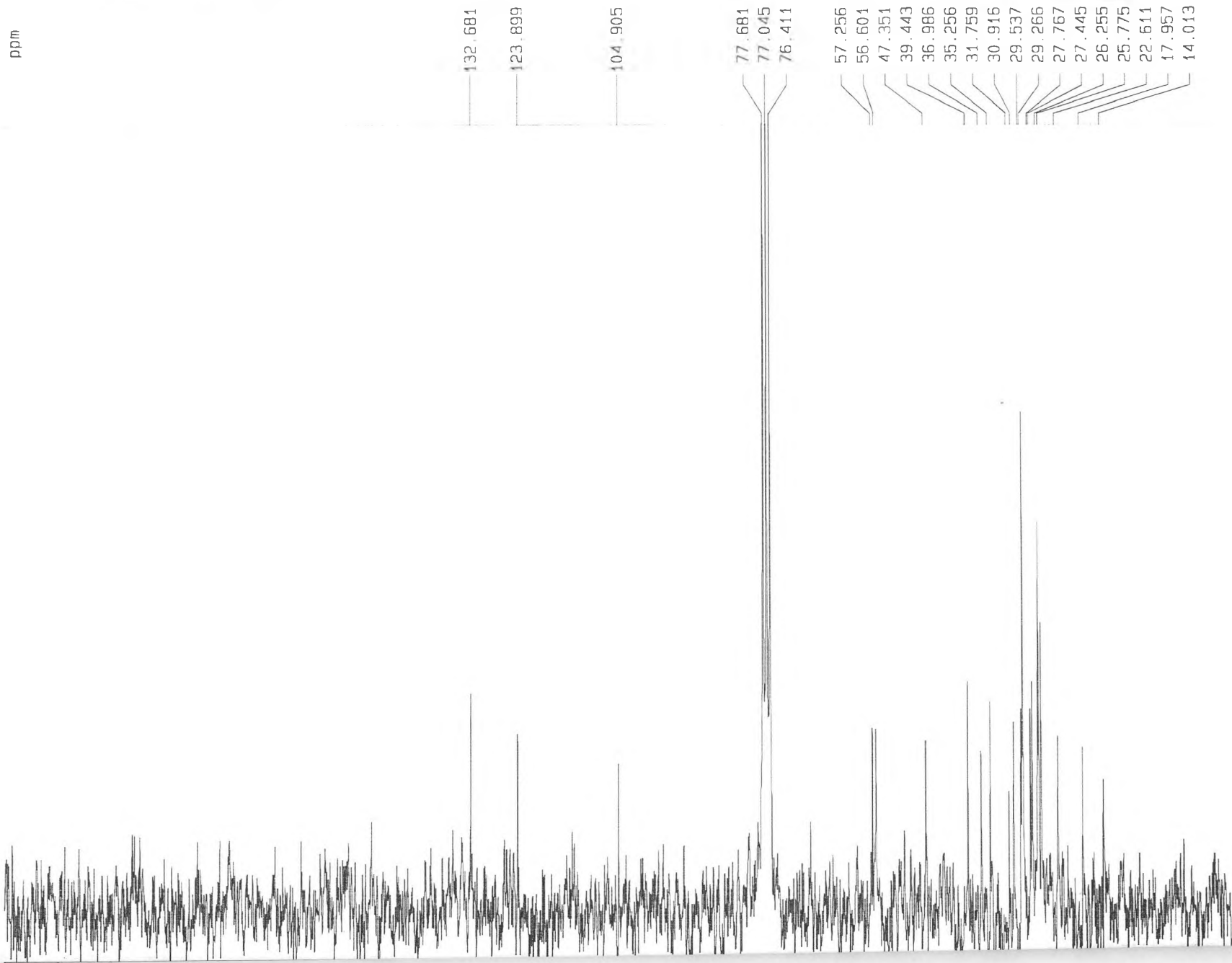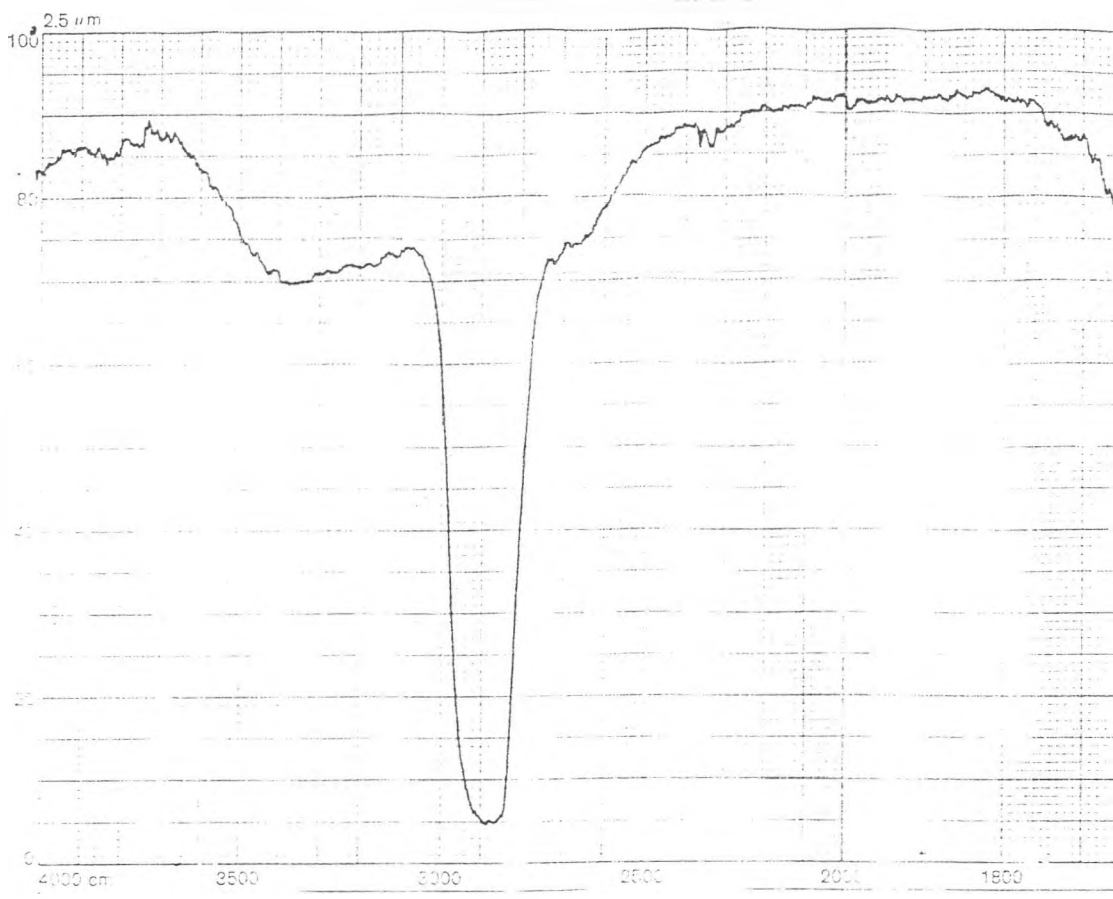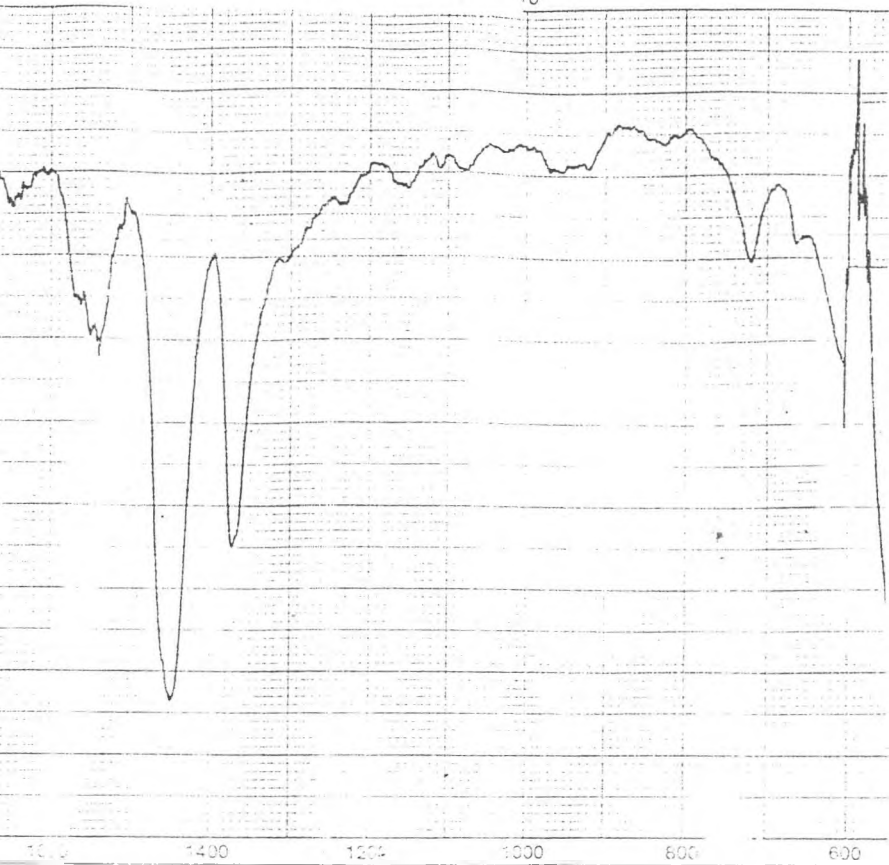
7.5 — -0.0238

7.0
6.5 — 0.0190

6.0

5.5 — 0.0644

5.0

4.5

4.0

3.5 — 0.3502

3.0

2.5 — 0.4762

2.0

1.5 — 1.0145

1.0 — 0.1215

0.5

0.0 — 0.0213

(ppm)

7.2400

7.0118
6.7154
6.6934
6.6715

6.0004
5.5319
5.5039
5.4892
5.4612
5.4331
5.4233
5.3953
5.3672
5.3526
5.3245

3.3919
3.3639
3.3358
3.3175
3.2931
3.1882
3.1638
3.1394
3.1150
2.9307
2.9137
2.8954
2.8746
2.8588
2.4244
2.3939
2.3598
2.3293
2.3036
2.2780
2.1877
2.1584
2.1255
2.0901
2.0633
2.0328
2.0157
2.0023
1.9889
1.9693
1.6875
1.6619
1.6375
1.6082
1.5899
1.4960
1.4606
1.4203
1.2751
0.8981
0.8713
0.8420
-0.0340

ppm

132.681
123.899
104.905
77.681
77.045
76.411
57.256
56.601
47.351
39.443
36.986
35.256
31.759
30.916
29.537
29.266
27.767
27.445
26.255
25.775
22.611
17.957
14.013

60

1600   1400   1200   1000   800   600
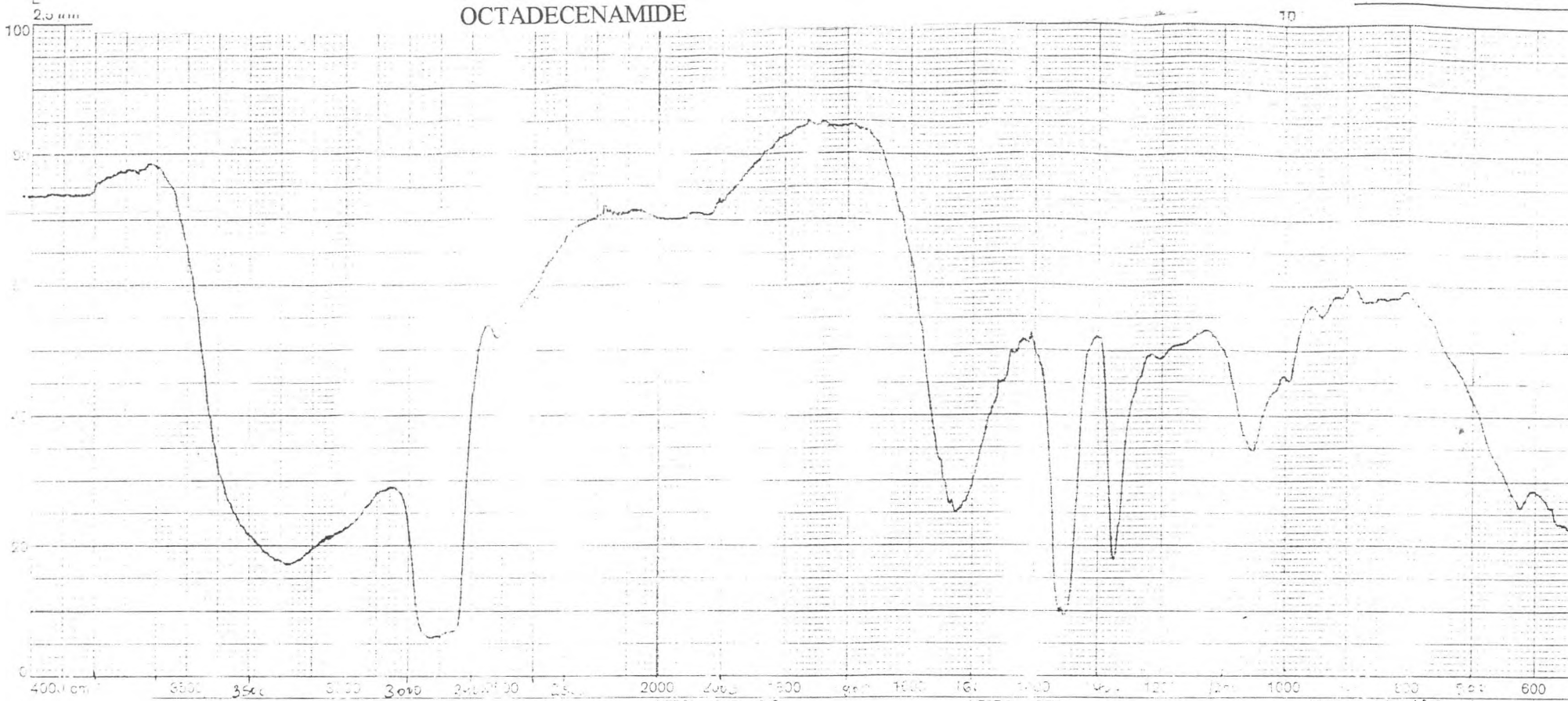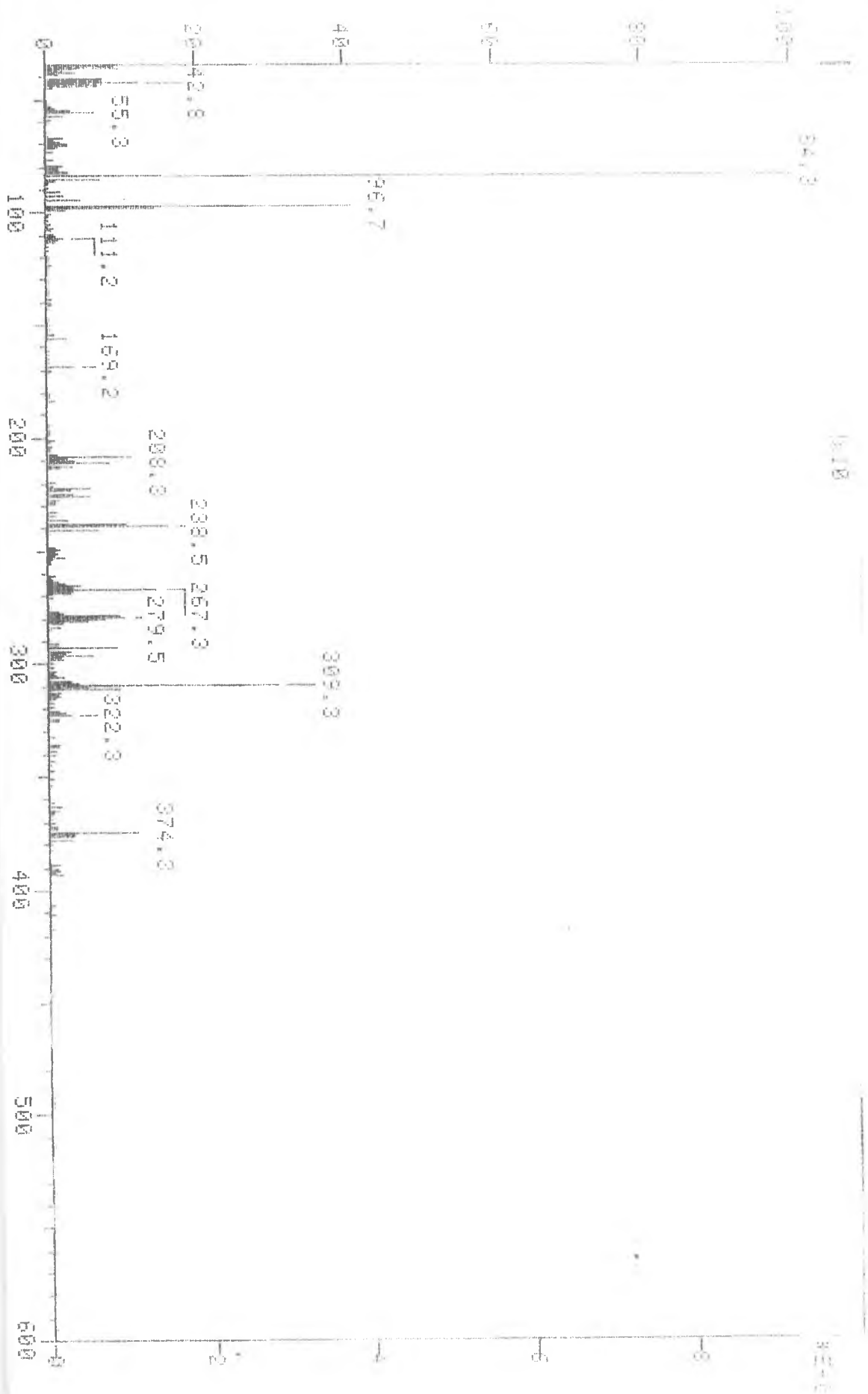
OCTADECENAMIDE

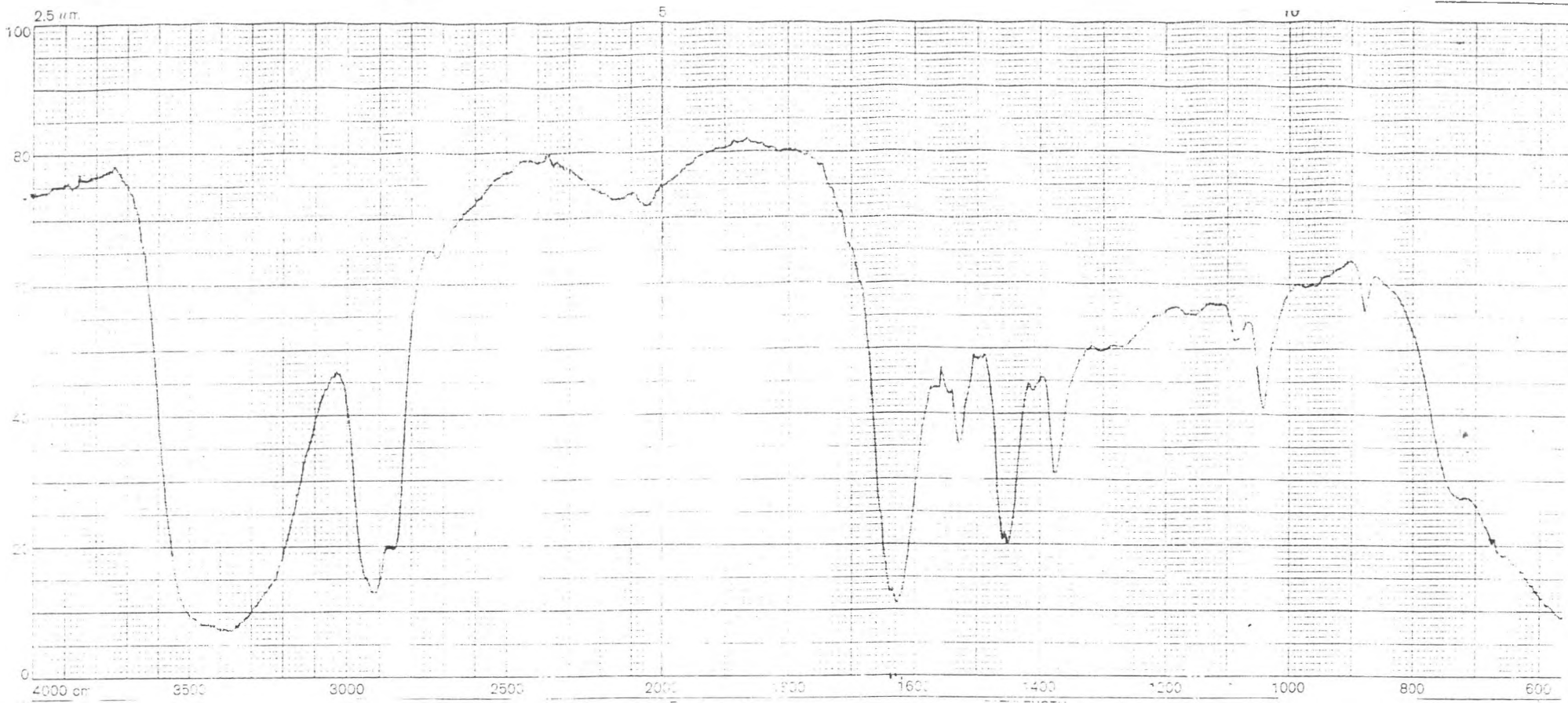62

FIG. 19 – EI MS OF 12, 13- DIHYDROXY-N-2-(1-PYRROLIDINO)ETHYL-9-OCTADECENAMIDE

FIG. 21 – EI MS OF THE HYDROXY ESTER