CHEMICAL INVESTIGATION ON THE ESSENTIAL OILS OF *SATUREJA ABYSSINICA* AND *S. PUNCTATA*

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By
Solomon Teklu
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School of Graduate Studies

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by

Solomon Teklu

Chemistry Department

Science Faculty

Apporved by:	**Signature**
Dr Gizachew Alemayehu Advisor	
Prof. S.E. Drewes External examiner	
Dr Ermias Dagne Examiner	
Dr Wendimagegn Mammo Examiner	
Dr Dirshaye Memberu Examiner	

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ABSTRACT

A study on the essential oils of *S. abyssinica* (Benth.) Briq. and *S. punctata* (Benth.) Briq. was undertaken.

The oil of *S. abyssinica* was analyzed and four components have been identified. Two compounds, pulegone and piperitenone were isolated and characterized. Pulegone (70-79%) was found to be the major constituent of the oil.

The oil of *S. punctata* was also analyzed and ten components have been isolated and identified. These include citral a and b, geranyl acetate, ß-caryophyllene, 3,7,10(15)-germacratriene, nerolidol, caryophyllene oxide and α-bisabolol. Citral a (40-43%) and citral b (30-33%) were found to be the major constituents of the oil. To the best of our knowledge, 3,7,10(15)-germacratriene is a novel natural product. All the other compounds are known and citral a is reported as a major constituent of *Satureja* species for the first time. *S. punctata* is placed into a new grouping called "Monoterpenic aldehyde type". Literature survey showed that there is no previous work on the chemical investigation of *S. abyssinica* and *S. punctata*.

The elucidations of the structures are based on spectroscopic techniques and by comparison of these data with those reported in the literature.

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1. INTRODUCTION

1.1 Essential oils

Essential oils, obtained by steam distillation of plants, are complex mixtures of odorous and steam volatile compounds which are deposited by plants in the subcuticular space of glandular hairs, in cell organelles, in idioblasts, in excretory cavities and canals of heartwoods [1].

Essential oils are used in the manufacture of perfumes, cosmetics, and toilet soaps [2]; as flavouring materials in candy, chewing gum, ice-cream, and for flavouring alcoholic as well as nonalcoholic beverages [3]. Still others have therapeutic or bactericidal properties and are valuable in medicine and dentistry [4].

The functions of the essential oils in plants are still not satisfactorily explained. Whether they serve as attractants, repellents, or protectants or are simply waste products is obscure [5].

1.1.1 Composition of essential oils

The principal constituents of essential oils are the terpenes. Benzenoid and aliphatic compounds may also be present. Most of the constituents are hydrocarbons and oxygenated derivatives of hydrocarbons. A few contain nitrogen and sulfur. For example, oil of mustard contains organic isothiocyanates; garlic and onion oils contain organic sulfides [6].

Of the terpenes, the monoterpenes and sesquiterpenes are the most abundant components of essential oils. In some essential oils, a terpene is the major constituent. For example, bois de rose oil contains about 85% linalool, palmarosa about 84-90% geraniol, and lemon-grass oil over 75% citral [6]. It is frequently advantageous to isolate these major constituents from the oils for perfume and flavour applications, as well as to convert them to other important derivatives.

Other essential oils contain large amounts of nonterpene components. Methyl salicylate makes up about 98% of oil of sweat birch, anethole about 90% of anise oil, and eugenol as much as 95% of clove oil [7].

Most essential oils are exceedingly complex mixtures of terpene and nonterpene ingredients. For example, 75 compounds have been isolated from camphor oil [6].

1.1.2 Methods of production of essential oils

The most common method of separating the essential oils from plant materials is steam distillation. In order to recover the maximum amounts of oil, the plant material is either crushed or comminuted.

Unique processes have been developed for almost every oil. The flavour oils in the citrus fruits are held in tiny sacs in the rind. They are mechanically ruptured and the oils collected by decantation, centrifugation and filtration. The oils of orange, lemon, mandarin and bergamot are obtained commercially in this way. Because the oils from blossoms such as jasmine, gardenia, tuberose and violet are destroyed or lost during steam distillation, a process termed enfleurage has been used. The blossoms are placed in contact with purified fats, which become saturated with the odorous oils. Extraction with alcohol then removes the oils from the fats. In some cases plant materials are extracted by volatile solvents such as petroleum ether and benzene. Such extractions remove not only most of the perfume materials but also waxes, colouring matter and resinous materials. The solvents are removed, and the residue is frequently reextracted with alcohol to obtain oils of higher purity. Some flower oils are treated in this way, as are olibanum, myrrh, opopanax, ginger, capsicum and celery.

1.1.3 Analysis and Synthesis of essential oils

Chemical, physical and organoleptic (odour or taste) analyses serve to determine the quality of the oils. There are many different methods used to evaluate the essential oils and perfumes. Paper chromatography, infrared spectroscopy, and vapour fractometry play significant roles in evaluating the essential oils and perfumes. All the methods

possess limitations, and odour and taste must maintain the final criteria, since most end uses take advantage of these qualities of the oils [6].

No essential oil has been successfully reconstructed. Synthetic methyl salicylate for instance can be differentiated by odour and taste from oil of sweet birch even though the natural oil contains 98% methyl salicylate. Synthetic methods have made possible the commercial production of important constituents of essential oils, such as linalool, linalyl acetate, nerolidol, citral, ß-ionone, geranyl acetate, nerol and α-irone [6].

1.2 Biogenesis of terpenoids

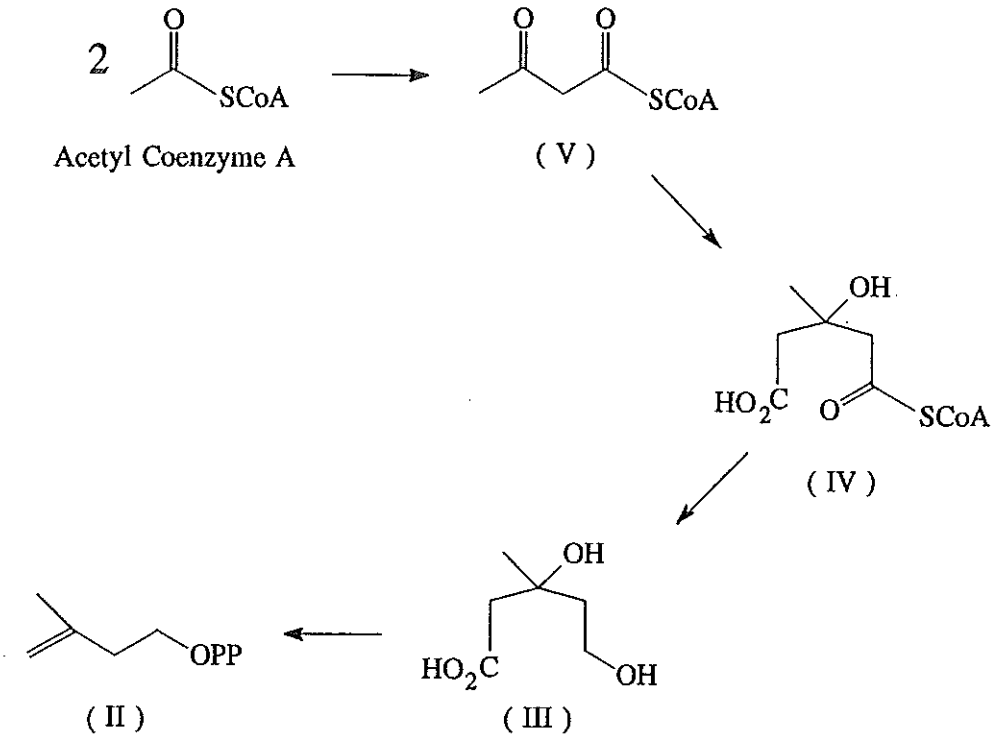
Metabolism of acetate gives rise to a large and structurally very diverse group of secondary metabolites : the *isoprenoids* or terpenoids.

1.2.1 Biogenesis of isopentenyl pyrophosphate

It soon became apparent that the terpenoids possessed a common structural feature : they contained an integral number of C_5 units. Furthermore, isoprene, 2-methylbuta-1,3-diene, was often obtained on pyrolysis of these C_{10} compounds and it was suggested that isoprene was the building block for terpene biosynthesis : condensation of successive isoprene units in a head-to-tail fashion would produce compounds of formula $(C_5)_n$. This was known as "the isoprene rule", and hence the term isoprenoids.

All terpenoids can be derived from an isoprene unit in the form of isopentenyl pyrophosphate which serves as a nucleus for the condensation of further 5-carbon units. The key building block, isopentenyl pyrophosphate (II), arises from mevalonic acid (III) *via* hydroxymethylglutarate (IV), (Scheme 1) [8].

The starting point of this metabolic pathway is believed to be the condensation of two molecules of acetic acid to form acetoacetyl coenzyme A (V). This follows an essentially irreversible (probably rate-limiting) two-step reduction *via* hydroxymethylglutarate (IV) to produce mevalonic acid (III). Mevalonic acid is then phosphorylated, and MVA-5-pyrophosphate is decarboxylated and dehydrated to yield



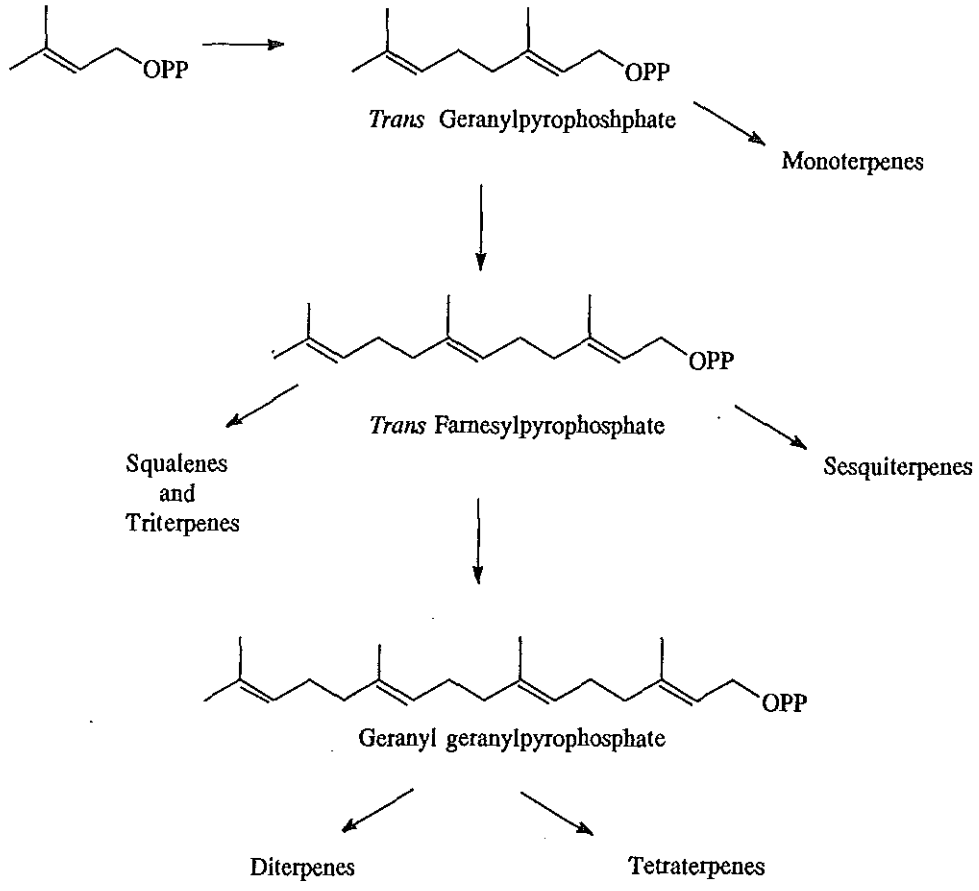
Scheme 1 : Biogenesis of isopentenyl pyrophosphate

isopentenyl pyrophosphate. Isopentenyl pyrophosphate is then readily converted to terpenes by polymerization in a variety of ways (Scheme 2) [9].

1.2.2 Biogenesis of monoterpenes

Acyclic or cyclic C_{10} hydrocarbons and their oxygenated derivatives are known as monoterpenes. The first stage in the formation of monoterpenes is the linking of isopentenyl pyrophosphate (II) and dimethylallyl pyrophosphate (VI) to give geranyl pyrophosphate (IX) or neryl pyrophosphate (VIII) *via* the cation (VII) (Scheme 3) [5].

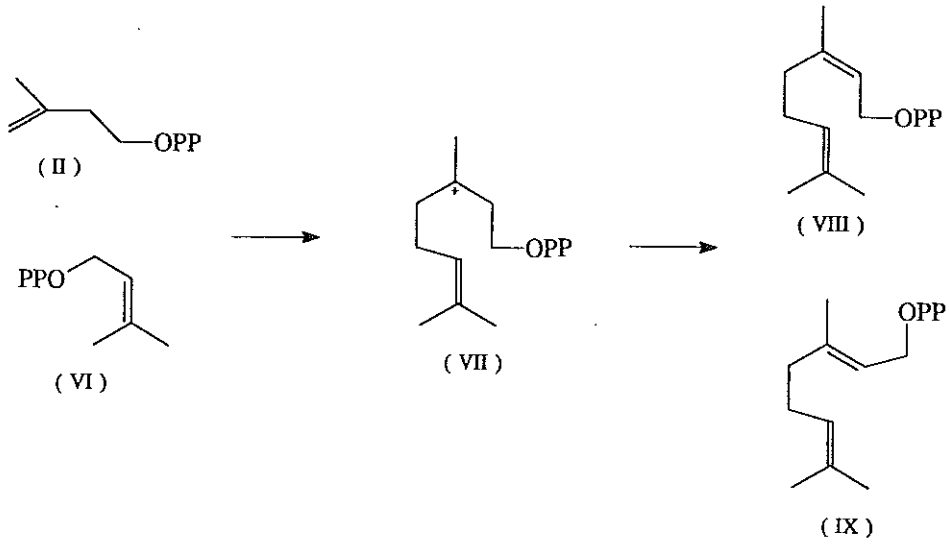
Alicyclic, monocyclic and bicyclic monoterpenes are found in many plants and in certain insects, but only rarely in animals. Enzyme studies have shown that both acyclic and cyclic compounds can be derived from geranyl pyrophosphate (GPP) and



Scheme 2 : Biogenesis of terpenoids

from neryl pyrophosphate (NPP) (Scheme 4) [10].

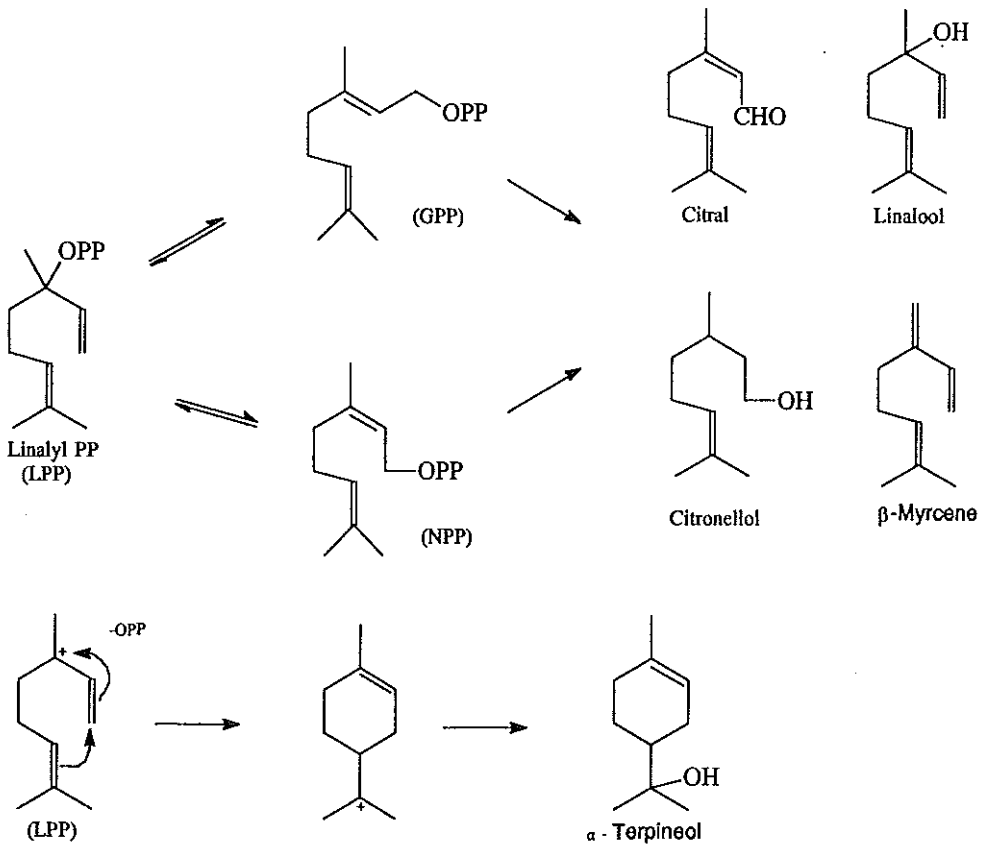
Monoterpenes are widely used in the flavour and perfume industries because of their attractive odours, low molecular weights, and high volatilities [8]. Most are synthesized rather than extracted from plant sources.



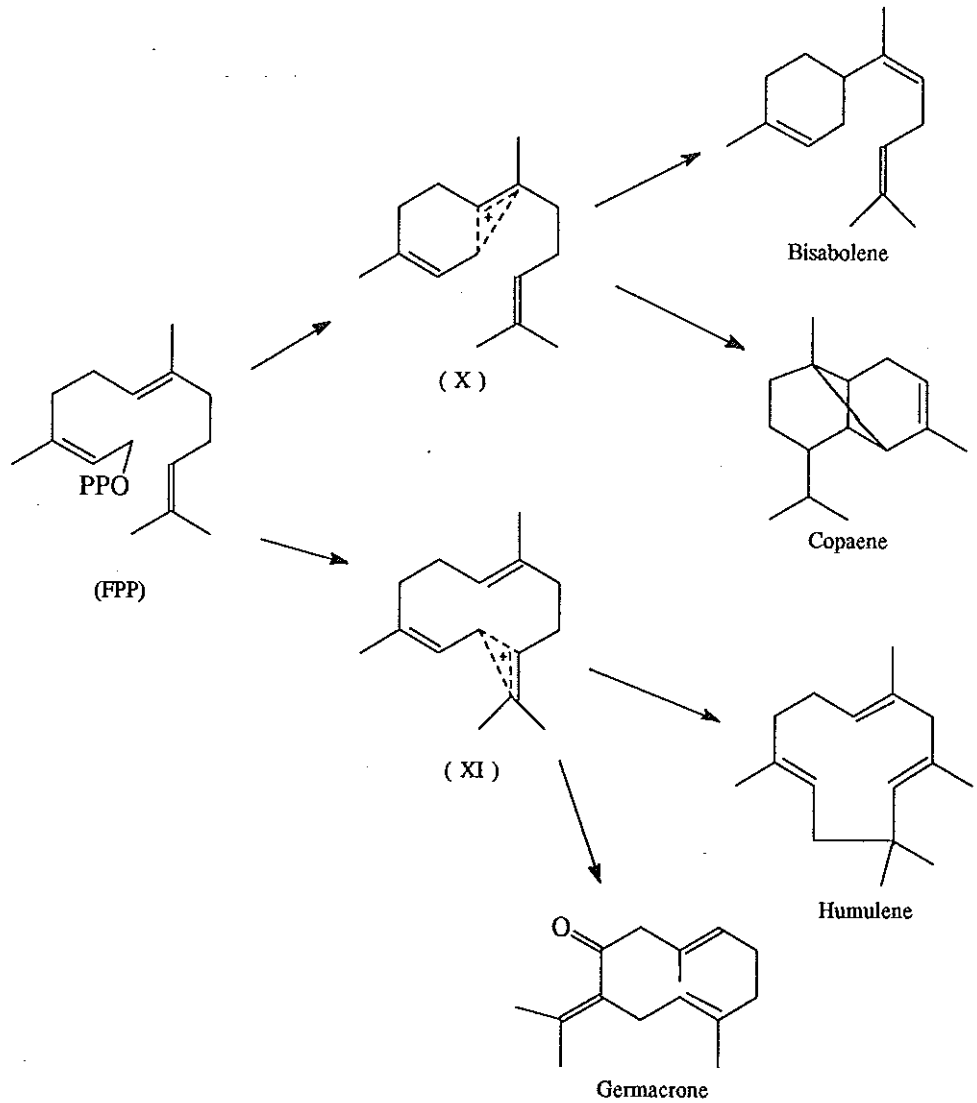
Scheme 3 : Biogenesis of geranyl pyrophosphate and neryl pyrophosphate

1.2.3 Biogenesis of sesquiterpenes

These are C_{15} hydrocarbons or their oxygenated analogues. As a family, the sesquiterpenes encompass an almost bewildering array of structural types and more than fifty basic skeletons have been recognized. They arise from the cyclization of farnesyl pyrophosphate (FPP) and subsequent rearrangements of the resulting carbonium ions (X, XI) (Scheme 5) [5, 11].



Scheme 4 : Biogenesis of monoterpenes



Scheme 5 : Biogenesis of sesquiterpenes

1.3 Genus *Satureja*

The genus *Satureja*, in the family Labiatae has about 30 species distributed in tropical Africa, Europe and North America [12]. In Ethiopia, there are eight *Satureja* species namely, *S.abyssinica* (Benth.) Briq., *S. biflora* (Buch. Ham. ex Don) Briq., *S. kilimandschari* (Guerke) Hedb., *S. paradoxa* (Vatke) Engler, *S. pseudosimensis* Brenan, *S. pseudosimensis* Var. *micrantha* Cufod., *S. punctata* (Benth.) Briq. and *S. simensis* (Benth.) Briq. [13].

The herbs of *Satureja* species can be cultivated for condiments. The fragrant leaves and dried leaves of the species are used as tea and spice, as flavouring like sage often in "mixed herbs" and they are traditionally used with legumes especially broad beans [12]. Some species of *Satureja* may also be added to medicines for their aromatic and warming qualities. They were formerly deemed sovereign remedies for colic and cure for flatulence, on this account, and were also considered good expectorants [14].

1.4 *Satureja abyssinica*

S. abyssinica (Syn. *Micromeria abyssinica* A.Rich., Vernacular names : *Etse meaza*, Gz.; *Yelomi eshet, Mutansa*, Am.), being one of the species of the genus *Satureja*, is an undershrub or perennial shrubby herb, strongly aromatic and can be up to 20-80 cm high with corolla pink to pale blue flowers. It grows in Ethiopia on dry hillsides, slopes of river banks, eroded red soil, loose clay soil, exposed rocky slopes with scattered *Eucalyptus globulous* or associated grassy area at 1400-2460 m in Shoa, Bale, Sidamo, Arssi, Keffa, Gojam, Gondar, Tigray and Harerge regions. It also grows in Eriteria, Kenya, Uganda and Tanzania [15]. The plant's infusion in hot water is used for bathing and washing at the time of circumcision. The leaves are used for indigestion. Ash from burned leaves is licked for coughs [16]. The plant is also used to flavour tea and different kinds of *Wot* [15].

1.5 *Satureja punctata*

S. punctata (Syn. *Micromeria punctata* Benth.) is an erect perennial herb having purple (sometimes violet) flowers with a pleasant fragrance, aromatic and can be up to 30-80 cm high. It grows in Ethiopia on dry and often on rocky ground, highly grazed grassland occurring at altitudes of 1800-3700 m in Shoa, Gondar, Tigray, Wello, Gojam, Gamogofa, Bale, Sidamo, Harerge, Arssi and Keffa Regions. The plant also occurs in tropical Africa and S.Africa [15]. The lemon-scented leaves of *S. punctata* are used to flavour fish dishes [15].

Analysis of the essential oils of *Satureja* species was started in 1917 [17]. Since then different species were analyzed at different times [18-29] and they are known to

elaborate terpenoids. Table 1 shows a summary of the constituents of essential oils of *Satureja* species.

1.6 Objectives of the project

The fact that essential oils obtained from the leaves and flowers of *Satureja* species have varied industrial applications (as flavouring materials, medicines, perfumes) [14] leads us to study the chemical constituents of the essential oils of *S. abyssinica* and *S. punctata*. No phytochemical study on *S. abyssinica* and *S. punctata* has been reported before this work and because of the local uses and pleasant fragrances of the plants, we decided to analyse their essential oils. The aim of this project was, therefore, to analyze the volatile constituents of the essential oils of *S. abyssinica* and *S. punctata* and determine the compounds that contribute to the sweet aroma and flavour of the plants.

Table 1 : Constituents of essential oils of different *Satureja* species

Compound	*S. boliviana*	*S. calamintha*	*S. eugenioides*	*S. hortensis*	*S. montana*	*S. mountain*	*S. nepeta*	*S. obovata*	*S. odora*	*S. parvifolia*	*S. parnassica*	*S. thymbra*
d-borneol						+						
carvacrol	+			+	+	+		+			+	+
l-carvone						+						
β -caryophyllene												+
cedrole							+					
p-cymene					+	+					+	
dihydrocuminic acid						+						
dihydrolippione										+		
dipentane					+							
eugenol	+											
geraniol	+		+									
d-isomenthone							+					
limonene									+			
linalool	+		+									
linalyl acetate			+									
lippione							+					
menthol	+					+						
l-menthone						+						
nerol	+											
piperitone										+		
pulegone	+	+					+		+			
l-terpineol						+						
thymol						+		+				+
reference	18	19	20	21	22	23	24	25	26	26	27	28, 29

+ present

2. RESULTS AND DISCUSSION

The leaves along with the flowers of *S. abyssinica* and *S. punctata* were subjected to hydrodistillation. The essential oils obtained from the plants were then subjected to further analyses as detailed below.

2.1 Yield and physical characteristics

The essential oil yield of *S. abyssinica* and *S. punctata* range from 0.430 to 0.704% and from 0.220-0.456%, respectively. The values for the physical characteristics can be seen from Table 2 and Table 3.

Table 2 : Yield and physical characteristics of essential oils from *S. abyssinica*

Sample code	Yield (% v/w)	Refractive index	Specific rotation	Specific gravity
A-a	0.587	1.4834	+34.96	0.9121
DM-a	0.430	1.4841	+35.05	0.9156
G-a	0.704	1.4831	+32.04	0.9021

Table 3 : Yield and physical characteristics of essential oils from *S. punctata*

Sample code	Yield (% v/w)	Refractive index	Specific rotation	Specific gravity
E-p	0.456	1.4900	-1.35	0.9210
G-p	0.252	1.4911	-1.41	0.9035
M-p	0.220	1.4892	-1.91	0.8859

2.2 Chemical composition

The essential oils obtained from the leaves and flowers of *S. abyssinica* and *S. punctata* were subjected to gas chromatographic analyses.

2.2.1 Essential oils from *S. abyssinica*

The essential oils of *S. abyssinica* collected from the different areas (A-a, DM-a and G-a) were analyzed separately in order to check their difference in composition. The chromatograms of the oils were found to be quite similar (Figure 1-3).

The oils derived from *S. abyssinica* constitute ca 25 components. The compounds with percent composition greater than 0.5 are not more than seven, comprising 90-96% of the oils. The composition of the oils were observed to be simple mixtures, dominated by one compound which was identified to be pulegone (**1**) (Table 4 and 5).

Table 4 : Compounds identified from the essential oils of *S. abyssinica*

RT on SE-54, min	Compound	Methods of identification
2.91	α-Pinene	RT, PE
3.27	Sabinene	RT, PE
5.75	Pulegone	RT, PE, IR, NMR
7.28	Piperitenone	RT, PE, NMR

RT = Retention Time

PE = Peak Enhancement

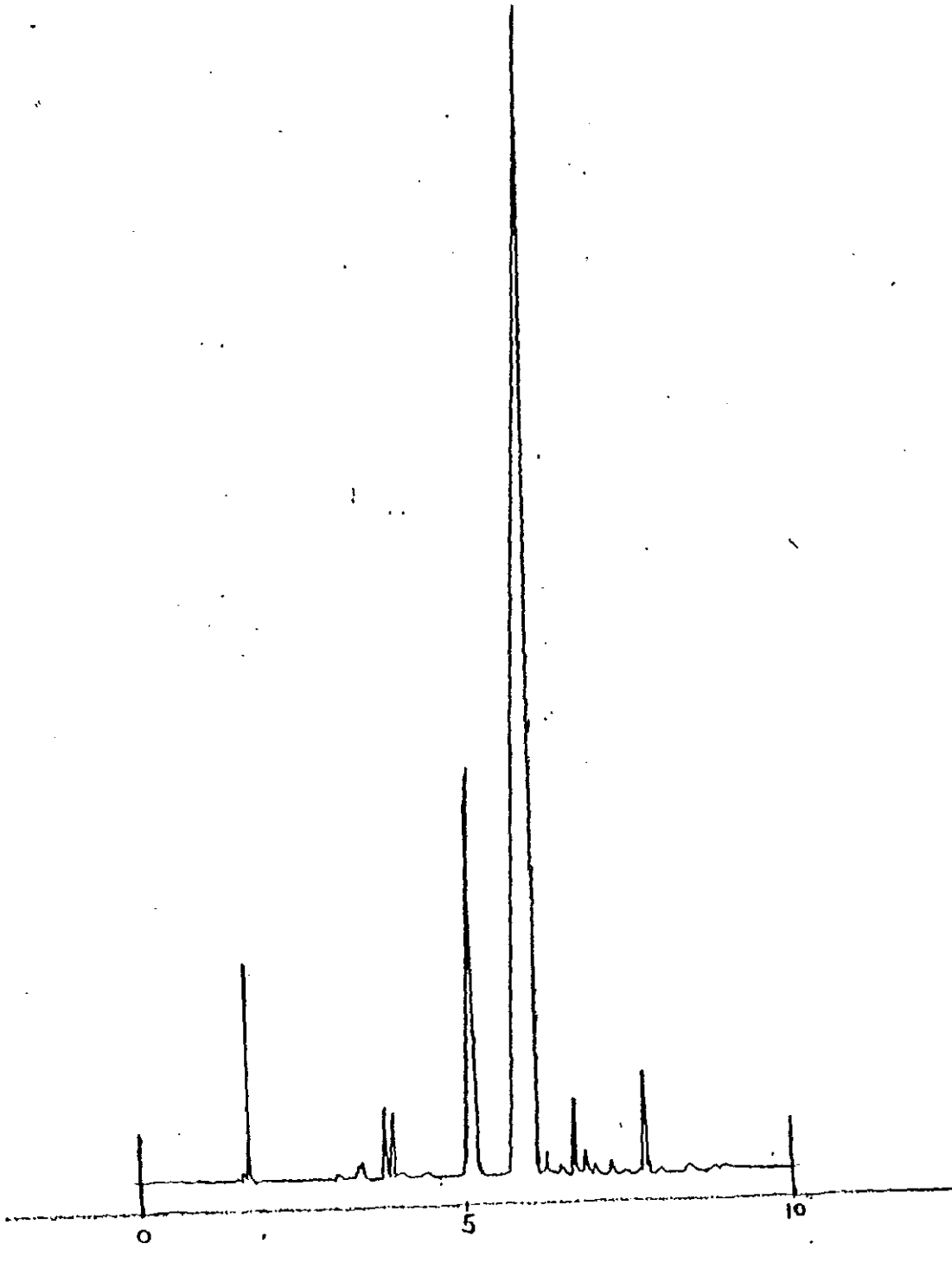


Figure 1 : Gas chromatogram of essential oil of *S. abyssini* (A-a)

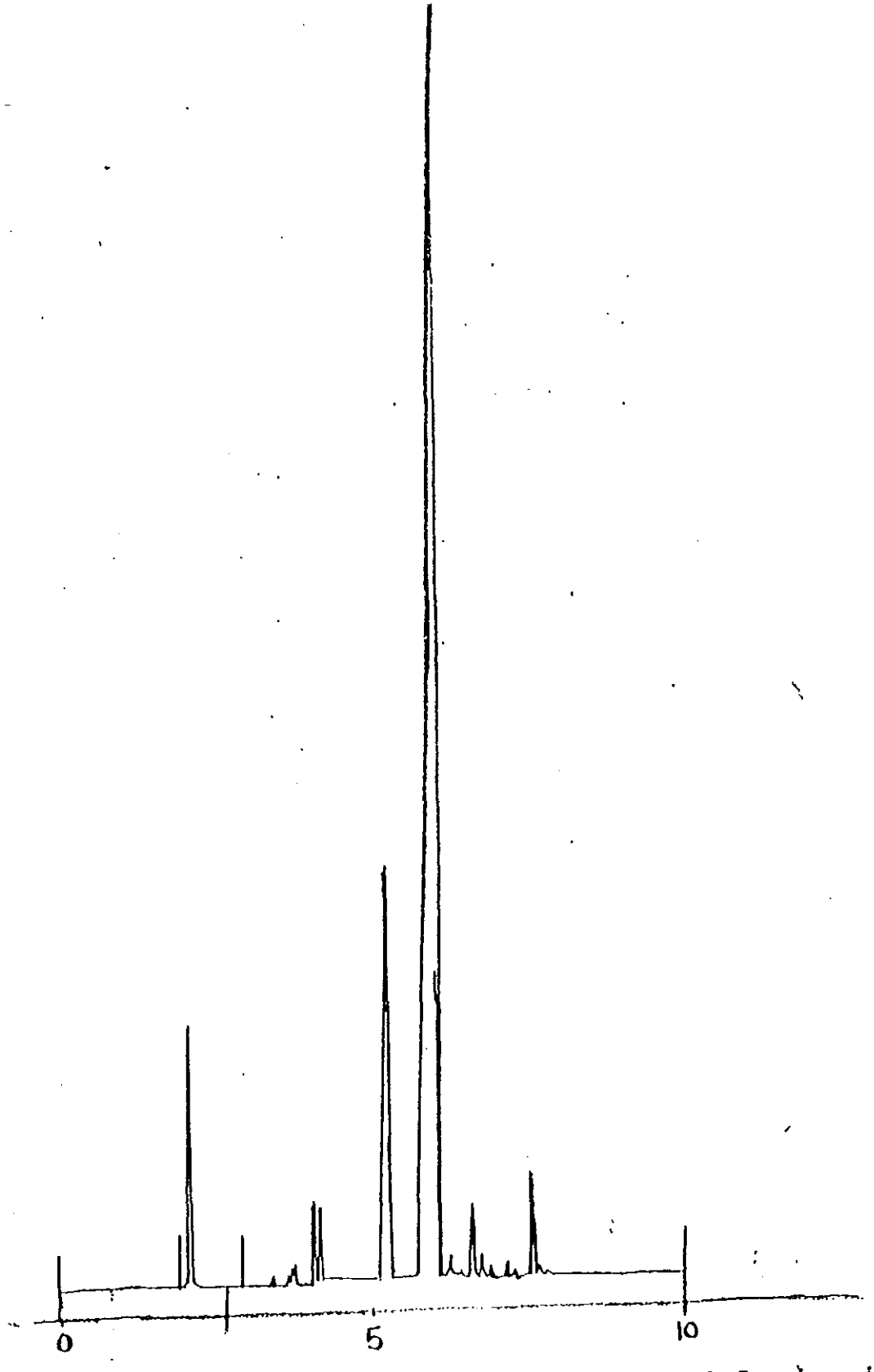


Figure 2 : Gas chromatogram of essential oil of *S. abyssinica* (DM-a)

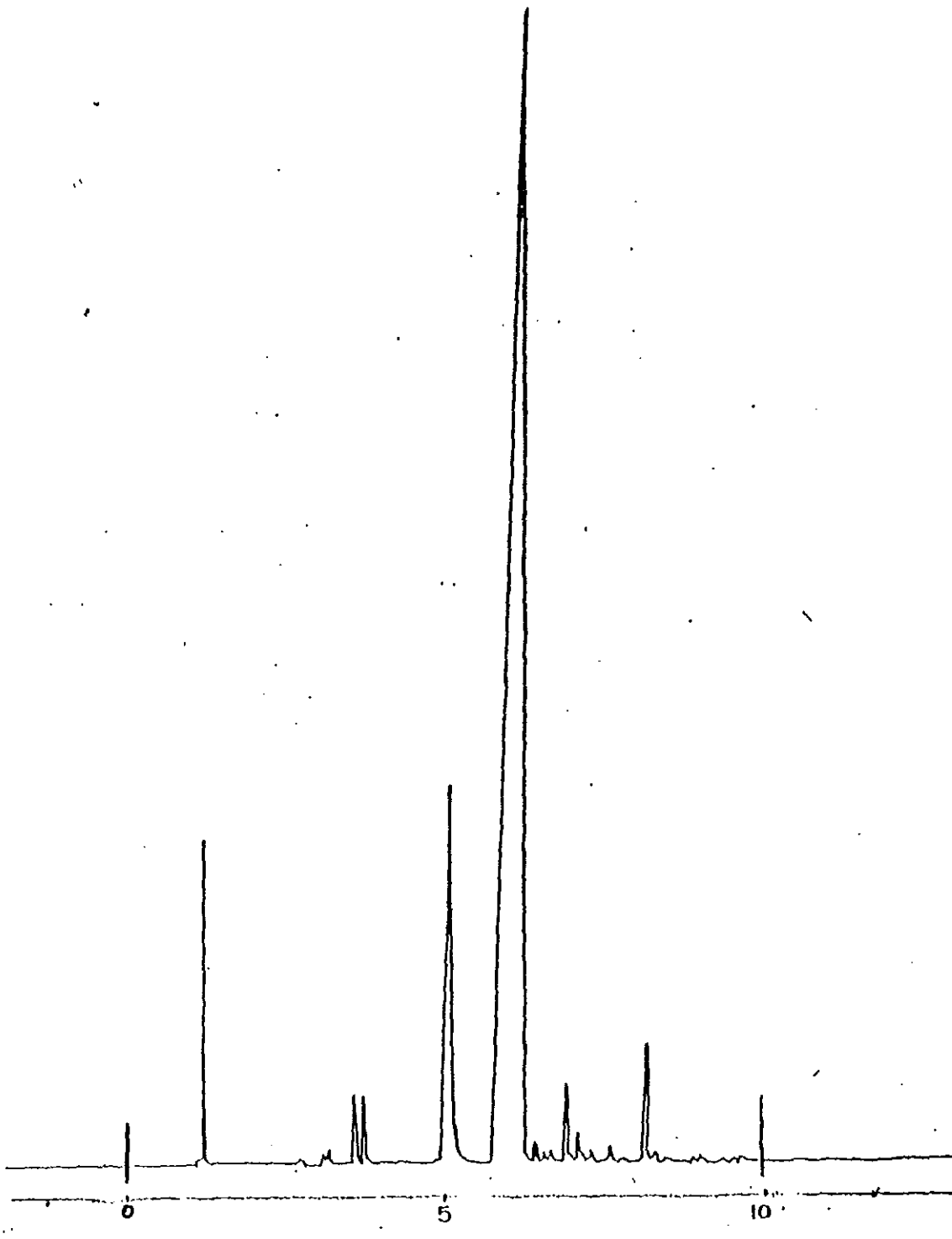


Figure 3 : Gas chromatogram of essential oil of *S. abyssini* (G-a)

Table 5 : Chemical composition of the essential oils of *S. abyssinica*

Compound	Percentage composition		
	A-a	DM-a	G-a
α-Pinene	0.26	0.10	0.06
Sabinene	1.19	0.72	0.25
Pulegone	69.52	74.33	79.37
Piperitenone	1.16	0.72	0.32

Pulegone comprising 69.52 to 79.37% of the oil was found to be the major component of oil of *S. abyssinica*. This monoterpene cyclic ketone was also found to be a major component in oils of *S. calamintha* [20], *S. nepeta* [25] and *S. odora* [26]. The pleasant peppermint odour of *S. abyssinica* is attributable to pulegone. Pulegone is used for scenting of soaps, but principally it serves as starting material for synthesizing menthol [30].

2.2.2 Essential oils from *S. punctata*

The relatively complex chromatograms of the essential oils derived from the leaves and flowers of *S. punctata* collected from different areas (E-p, G-p, M-p) were found to be quite similar (Figure 4-6). Each oil consists of *ca* 70 components and the compounds with percentage composition greater than 0.5 are about 15 and they comprised 94.58 to 95.62% of the oil. The composition of the oils was observed to be dominated by two compounds which were identified to be citral a (**4**) and citral b (**3**) (Tables 6 and 7).

Citral has a pleasant lemon odour, and finds extensive application as flavouring and perfumery ingredient. It is also an important starting material for the synthesis of ionones and vitamin A [31].

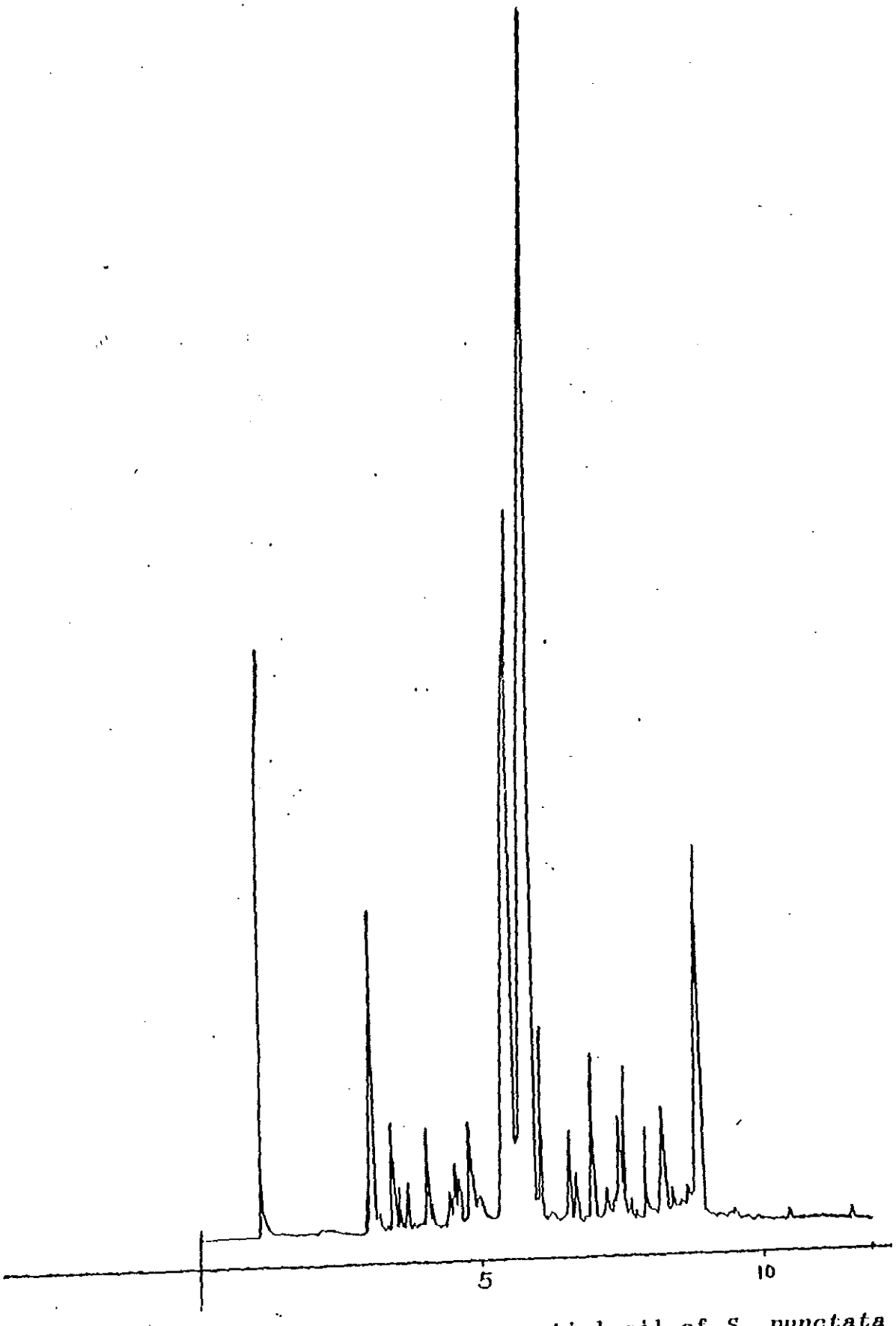


Figure 4 : Gas chromatogram of essential oil of *S. punctata* (E-p)

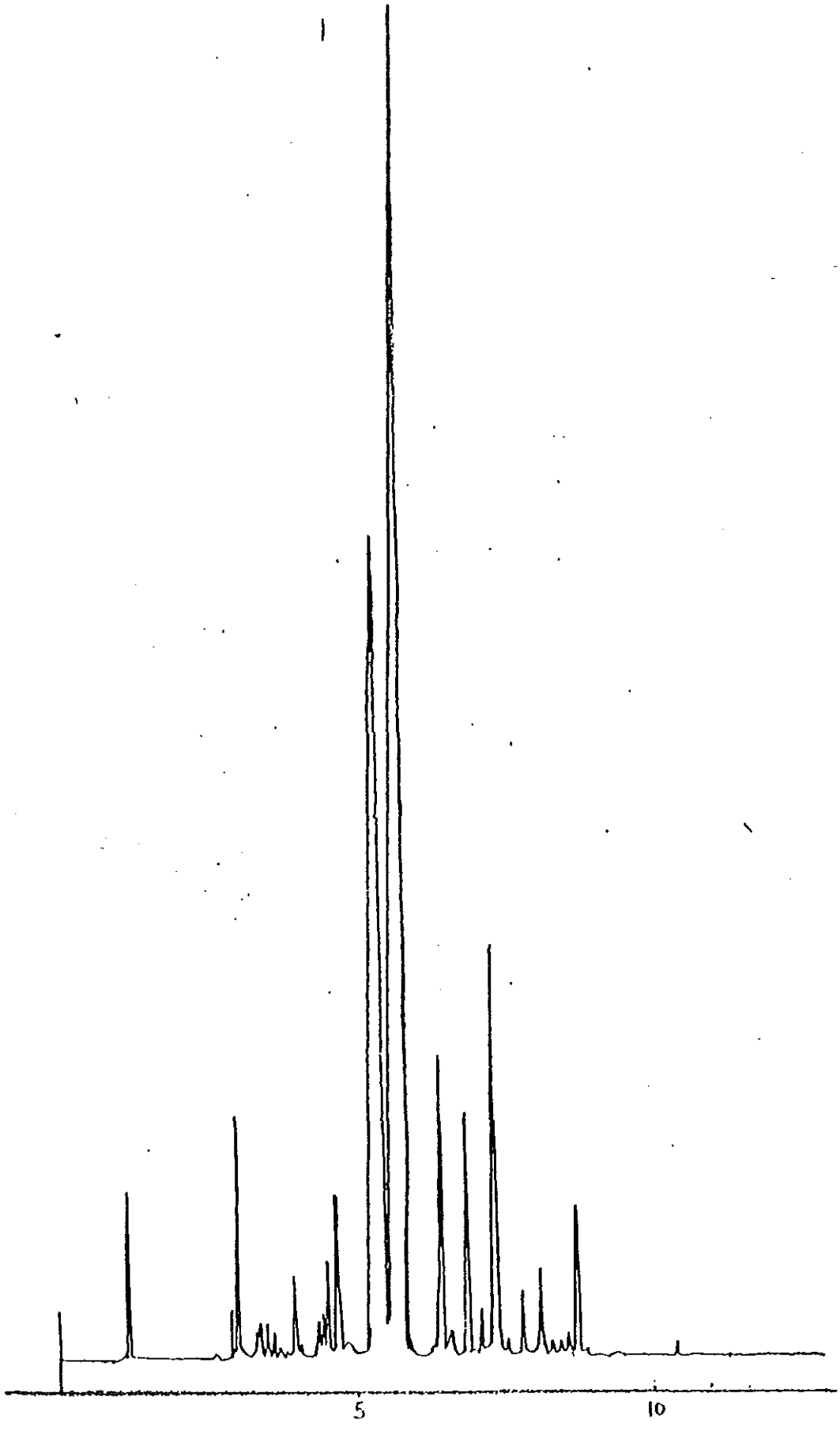


Figure 5 : Gas chromatogram of essential oil of *S. punctata* (G-p)

Figure 6 : Gas chromatogram of essential oil of *S. punctata* (M-p).

Table 6 : Compounds identified from the essential oils of *S. punctata*

RT on SE-54, min	Compound	Methods of identification
3.06	Sabinene	RT, PE
3.19	*β*-Pinene	RT, PE
5.56	Citral b	RT, PE, IR, NMR
5.93	Citral a	RT, PE, IR, NMR
6.59	Geranyl acetate	RT, PE, NMR
6.70	ß-Caryophyllene	IR, NMR
7.47	3,7,10(15)-Germacratriene	NMR
7.97	Nerolidol	RT, PE, IR, NMR
8.27	Caryophyllene oxide	RT, PE, NMR
8.94	*α*-Bisabolol	NMR

Table 7 : Chemical composition of the essential oils of *S. punctata*

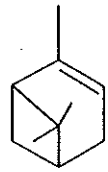
Compound	Percentage composition		
	E-p	G-p	M-p
Sabinene	0.30	0.39	0.28
β-Pinene	2.50	2.71	2.34
Citral b	33.58	30.25	31.72
Citral a	40.51	41.67	43.24
Geranyl acetate	1.25	1.01	1.35
ß-Caryophyllene	0.82	0.75	0.92
3,7,10(15)-Germacratriene	1.43	1.31	1.39
Nerolidol	1.11	1.31	1.09
Caryophyllene oxide	1.63	1.70	1.68
α-Bisabolol	6.78	6.27	5.98

Citral a constituting about 40 to 43% of the oil was found to be the major component of oil of *S. punctata*. The second major component comprising about 30 to 33% of the oil was citral b. The pleasant lemon odour of *S. punctata* is attributed to the presence of these compounds in large amount. Citral a has not been reported as a major component of any *Satureja* species before this work.

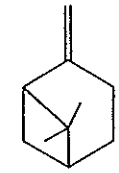
2.3 Isolation and characterization of the constituents

The essential oils were obtained from the plant materials by hydrodistillation using a clevenger apparatus. Isolation of compounds was conducted using column chromatography, chromatotron and preparative TLC, as described in the experimental section.

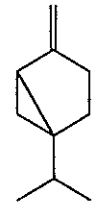
α-Pinene, sabinene and ß-pinene (Figure 7) were identified by comparison of their retention times with standard samples. Identifications of these compounds were further verified by adding a small amount of the standards into the oil and observing the growth of the suspected peaks on the chromatograms (Method of peak enhancement, PE).



α-Pinene



β - pinene



Sabinene

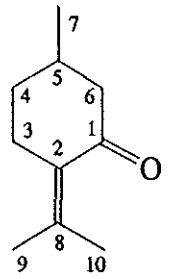
Figure 7 : Structures of *α*-pinene, ß-pinene and sabinene.

2.3.1 Pulegone (1)

This colourless monoterpene cyclic ketone was obtained from the ethyl acetate fraction of *S. abyssinica*. The IR spectrum displayed absorption bands at 1682 cm^{-1} and 1618 cm^{-1} that indicate the presence of a carbonyl and an olefinic groups respectively. The presence of methyl protons (δ 0.99), two methyl groups on a double bond (δ 1.75, 1.94), methine and methylene protons (δ 1.50-2.90) was evident from the ^1H NMR spectrum. The ^{13}C NMR with DEPT spectrum displayed 10 carbon resonances, corresponding to three CH_3 carbon atoms, three CH_2 carbon atoms, one CH carbon atom and three quaternary carbon atoms (Table 8).

Table 8 : ^{13}C NMR data of pulegone (**1**) (22.5 MHz, $CDCl_3$)

C	δ
1	203.7
2	131.8
3	32.7
4	28.5
5	32.0
6	50.7
7	21.6
8	141.9
9	22.1
10	22.9

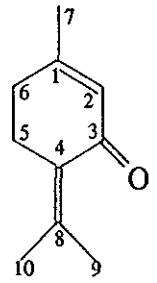


1

The above spectroscopic findings were found to be consistent with structure **1**. The identity of compound **1** was further established by comparison with an authentic sample using GC. The spectroscopic data obtained agree very well with that reported for pulegone in the literature [32, 33].

2.3.2 Piperitenone (2)

This colourless monoterpene cyclic enone was obtained from the ethyl acetate fraction of *S. abyssinica*. The ^{1}H NMR spectrum of this compound was found to be similar with that of pulegone except for the presence of a broad singlet at δ 5.85 suggesting the presence of an olefinic proton. The ^{1}H NMR spectrum of **2** revealed the presence of three methyl groups on double bonds (δ 1.83, 1.90, 2.08) and a proton on a trisubstituted double bond (δ 5.85). The above spectroscopic data led to the assignment of structure **2** to this natural product.



2

The identity of the compound was further confirmed by comparison with an authentic sample using GC. The spectroscopic data obtained agree very well with that reported for piperitenone in the literature [34, 35].

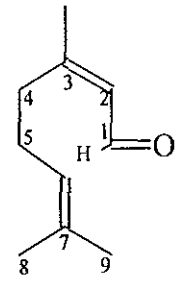
2.3.3 Citral b (3)

This compound was isolated from the EtOAc fraction of *S. punctata*. The IR spectrum has a band at 1674 cm^{-1} which indicates the presence of a carbonyl group. The presence of an aldehyde proton (δ 9.89), three methyl groups on double bonds (δ 1.60, 1.69, 1.98) and two protons on trisubstituted double bonds (δ 5.11, 5.85) was deduced from ^{1}H NMR. The ^{13}C NMR spectrum displayed 10 carbon resonances corresponding to three CH_3, two CH_2, three CH carbon atoms and two quaternary carbon atoms (Table 9).

The ^{1}H NMR and ^{13}C NMR data are consistent with structure **3** and agree very well with that reported in the literature [36, 37] for citral b. The identity of compound **3** was also established by comparison with an authentic sample using GC.

Table 9 : ^{13}C NMR data of Citral b (**3**) (22.5 MHz, $CDCl_3$)

C	δ
1	190.4
2	132.7
3	163.7
3-CH_3	26.1
4	32.5
5	26.3
6	122.7
7	132.7
8	17.7
9	24.6



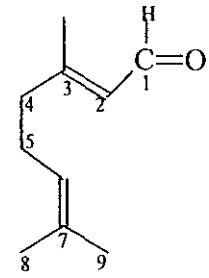
3

2.3.4 Citral a (4)

This compound was isolated from the EtOAc fraction of *S. punctata*. The IR spectrum showed a band at 1674 cm^{-1} which is indicative of the presence of a carbonyl group. The ^{1}H NMR spectrum indicated the presence of an aldehyde proton (δ 9.96), three methyl groups on double bonds (δ 1.60, 1.69, 2.18) and two olefinic protons on trisubstituted double bonds (δ 5.03, 5.85). The ^{13}C NMR spectrum displayed 10 carbon resonances, corresponding to three CH_3 carbon atoms, two CH_2 carbon atoms, three CH carbon atoms and two quaternary carbon atoms (Table 10).

Table 10 : ^{13}C NMR data of citral a (**4**) (22.5 MHz,$CDCl_3$)

C	δ
1	190.9
2	127.3
3	163.3
3-CH_3	25.5
4	40.6
5	25.8
6	122.6
7	132.8
8	17.5
9	17.6

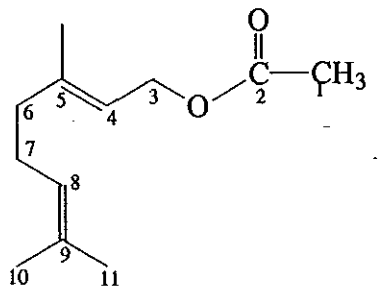


4

The identity of the compound was further confirmed by comparison with an authentic sample using GC. The ^1H NMR and ^{13}C NMR data are in very close agreement with that reported for citral a in the literature [36, 37].

2.3.5 Geranyl acetate (5)

This compound was isolated from the ethyl acetate fraction of *S. punctata*. The ^1H NMR spectrum indicated the presence of three methyl groups on double bonds and one methyl group on an acetate group (δ 1.65, 1.75, 2.01, 2.03), methylene protons adjacent to an acetate group (δ 4.52) and two olefinic protons on tri-substituted double bonds (δ 5.06, 5.35). The ^1H NMR data is consistent with structure **5** and agrees very well with that reported in the literature [38] for geranyl acetate.



5

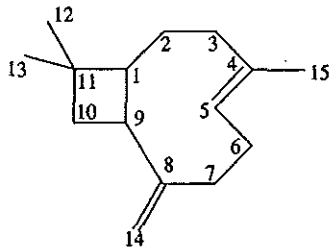
The identity of compound **5** was also established by comparison with an authentic sample using GC.

2.3.6 *β*-Caryophyllene (6)

This sesquiterpene hydrocarbon was isolated from the hexane fraction of *S. punctata*. The IR spectrum showed a band at 1634 cm^{-1} which is indicative of the presence of an olefinic group. The presence of two methyl groups (δ 0.92, 1.19), one methyl group on a double bond (δ 1.57), terminal methylene protons (δ 4.80, 4.91) and a proton on a trisubstituted double bond (δ 5.21) was evident from the ^{1}H NMR spectrum. The ^{13}C NMR spectrum, along with DEPT experiment, displayed 15 carbon resonances, corresponding to three CH_3 carbon atoms, six CH_2 carbon atoms, three CH carbon atoms and three quaternary carbon atoms (Table 11).

Table 11 : ^{13}C NMR data of ß-caryophyllene (**6**) (22.5 MHz, $CDCl_3$)

C	δ
1	48.5
2	28.3
3	40.3
4	134.6
5	124.3
6	34.8
7	39.9
8	154.6
9	53.5
10	29.3
11	32.9
12	22.6
13	16.3
14	111.6
15	30.0



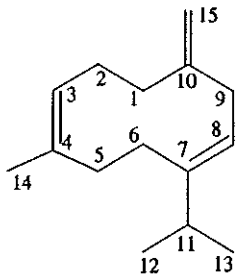
6

The ^1H NMR and ^{13}C NMR data are in very close agreement with that reported for ß-caryophyllene in the literature [39, 40].

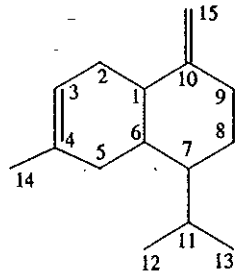
2.3.7. 3,7,10(15)-Germacratriene (7a)

This compound was isolated from the hexane fraction of the oil of *S. punctata*. The MS displayed a molecular ion peak at m/z 204. The base peak appeared at m/z 134. Other prominent peaks were at m/z 161, 132, 119, 109, 93, 81. The ^1H NMR data indicates the presence of an isopropyl group (δ 0.82, 0.89, both doublets, 6H), a methyl group on a double bond (δ 1.60), saturated methylene and methine protons (δ 1.20-2.00), terminal methylene protons (δ 4.75, 4.79) and two protons on trisubstituted double bonds (δ 5.15, 5.40). The ^1H NMR data has a close similarity

with that of γ_2-cadinene (3,10(15)-cadinadiene) (**7b**), a constituent of oil of *Vetiveria zizanioides* [41] (Table 12).



7a

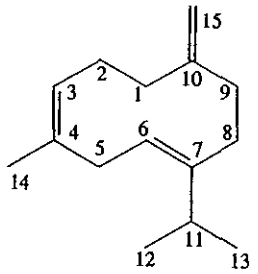


7b

Table 12 : ^{1}H NMR data of 3,7,10(15)-germacratriene (**7a**) and γ_2-cadinene (**7b**) (300 MHz, $CDCl_3$)

Proton	3,7,10(15)-germacratriene	γ_2-cadinene
H-3	5.40	5.48
H-7	-	1.20-2.00
H-8	5.15	1.20-2.00
H-12	0.82	0.73
H-13	0.89	0.93
H-14	1.60	1.67
H-15	4.75	4.57
H-15′	4.79	4.69

However, an extra resonance was observed at δ 5.15 for 3,7,10(15)-germacratriene. This suggests the presence of an olefinic proton which is absent in γ_2-cadinene (**7b**). The position of the double bond should be between either C-7 and C-8 (**7a**) or C-6 and C-7 (**7c**) to explain the triplet observed at δ 5.15. According to the present spectroscopic data, it is not possible to distinguish between the structures **7a** and **7c**. Further spectroscopic data need to be obtained to reach to one of the structures.



7c

The ^{13}C NMR spectrum of 3,7,10(15)-germacratriene (Table 13) shows a clear difference between the two compounds and revealed 15 carbon resonances, corresponding to three CH_3 carbon atoms, six CH_2 carbon atoms, three CH carbon atoms and three quaternary carbon atoms. From the structure of γ_2-cadinene (**7b**) however, there are three CH_3 carbon atoms, five CH_2 carbon atoms, five CH carbon atoms and two quaternary carbon atoms.

Table 13 : ^{13}C NMR data of 3,7,10(15)-germacratriene (**7a**) (22.5 MHz, $CDCl_3$)

C	δ	C	δ
1	34.5	9	31.1
2	26.7	10	154.2
3	123.9	11	40.0
4	131.1	12	17.5
5	30.3	13	23.1
6	28.1	14	25.6
7	133.6	15	107.0
8	120.9		

The signal at δ 120.9 is assignable to the CH carbon atom (C-8). The signal at δ 133.6 is assigned to the quaternary carbon atom (C-7). The signals at δ 34.5 and 28.1 are respectively assigned to the CH_2 carbon atoms C-1 and C-6. Since there are six CH_2

carbon atoms in 3,7,10(15)-germacratriene, the two CH carbon atoms (C-1 and C-6) in γ_2-cadinene should be converted to CH_2 carbon atoms by braking the bond between C-1 and C-6.

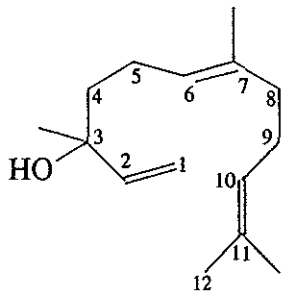
The above spectroscopic data allowed the assignment of structure **7a** or **7c** to this compound. The properties of this compound did not correspond with those of any known sesquiterpene hydrocarbon, in particular with helminthogermacrene (1(10),5,11-germacratriene) [42], and germacrene A (1(10),4,11-germacratriene) [43]. To the best of our knowledge, compound **7a** or **7c** is a novel natural product, hence thename3,7,10(15)-germacratriene(7-isopropyl-4-methyl-10-methylenecyclodeca-3,7-diene)or3,6,10(15)-germacratriene(7-isopropyl-4-methyl-10-methylenecyclodeca-3,6-diene) has been adopted to this new compound.

2.3.8 Nerolidol (8)

This sesquiterpene alcohol was isolated from the EtOAc fraction of *S. punctata*. The ^{1}H NMR spectrum indicated the presence of one methyl group on an asymmetric carbon (δ 1.20), three methyl groups on double bonds (δ 1.51, 1.68), terminal methylene protons (δ 4.95), a proton on an -O<u>H</u> group (δ 5.09) and protons on tri-substituted double bonds (δ 5.09, 5.25, 5.91). The ^{13}C NMR spectrum showed the presence of 15 carbon resonances corresponding to four CH_3 carbon atoms, five CH_2 carbon atoms, three CH carbon atoms and three quaternary carbon atoms (Table 14). These spectroscopic data were found to be consistent with structure **8**.

Table 14 : ^{13}C NMR data of nerolidol (**8**) (75 MHz, $CDCl_3$)

C	δ
1	111.7
2	145.1
3	73.5
3-CH_3	25.7
4	42.1
5	39.7
6	124.3
7	135.6
7-CH_3	27.9
8	22.7
9	26.7
10	118.4
11	131.5
11-CH_3	16.1
12	17.7

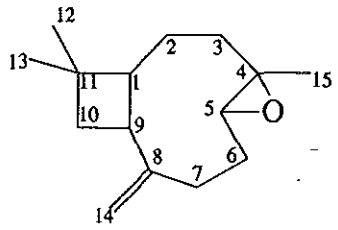


8

The ^1H NMR data of **8** was similar with the data reported for nerolidol in the literature [44]. The identity of **8** was also confirmed by comparison with an authentic sample using GC.

2.3.9 Caryophyllene oxide (9)

This sesquiterpene oxide was isolated from the EtOAc fraction of *S. punctata*. The ^1H NMR spectrum of this compound was found to be similar to that of β-caryophyllene except the absence of a broad singlet at δ 5.21 indicating the presence of an olefinic proton. The presence of two methyl groups (δ 0.94, 1.16), one methyl group on an asymmetric carbon (δ 1.21) and terminal methylene protons (δ 4.86, 4.97) was evident from ^1H NMR.



9

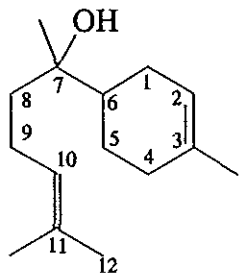
The spectroscopic data was found to be consistent with that reported for caryophyllene oxide in the literature [45]. The identity of **9** was also established by comparison with authentic sample using GC.

2.3.10 α-Bisabolol (10)

This sesquiterpene alcohol was isolated from the EtOAc fraction of *S. punctata*. The presence of a methyl group on an asymmetric carbon (δ 1.10), three methyl groups on double bonds (δ 1.62, 1.65, 1.68), methine and methylene protons (δ 1.20-2.01), a proton on an -OH group (δ 2.01) and two olefinic protons on trisubstituted double bonds (δ 5.13, 5.38) was evident from ^{1}H NMR. The ^{13}C NMR spectrum displayed 15 carbon resonances corresponding to four CH_3 carbon atoms, five CH_2 carbon atoms, three CH carbon atoms and three quaternary carbon atoms (Table 15).

Table 15 : ^{13}C NMR data of α-bisabolol (**10**) (75 MHz, $CDCl_3$)

C	δ
1	31.1
2	124.5
3	134.4
3-CH_3	26.7
4	23.2
5	22.1
6	42.9
7	74.1
7-CH_3	23.3
8	40.1
9	26.9
10	120.5
11	131.8
11-CH_3	17.7
12	23.2



10

The ^1H NMR and ^{13}C NMR data of **10** are found to be consistent with the data found in the literature for α-bisabolol [46, 47].

Numerous studies on the essential oils of *Satureja* species growing in the world exist in the literature [48 and references therein]. These studies show that, the genus *Satureja* may or may not contain phenols. Phenol-containing species are divided in to "carvacrol type" and "thymol type". Species not containing phenols are divided in to "Monoterpene alcohol type" and "Monoterpenic ketone type". Table 16 [48] shows a listing of *Satureja* spp. according to the major components of their oils. In the species listed the commonest type is that containing mainly monoterpene ketones in their essential oils followed by those containing carvacrol. *S. abyssinica* therefore belongs to the monoterpene ketone group in contrast to *S. punctata* whose major component is an aldehyde. *S. punctata* hence belongs to none of the groups listed in Table 16. This result may therefore lead to the insertion of a new monoterpenic type, "Monoterpenic aldehyde type" in the classification so far done (Table 16).

Table 16 : The main essential oil constituents of the *Satureja* genus.

Species	Carvacrol type	Thymol type	Monoterpenic alcohol type	Monoterpenic aldehyde type	Monoterpenic ketone type	Monoterpenic HC* type
*abyssinica***					+	
boliviana			+			
brownei					+	
calamintha					+	
cuneifollia	+					
douglasi					+	
granatensis	+					
gilliesii						+
horvatii		+				
hortensis	+					
innota					+	
kitaibelli					+	
montana		+	+			
obovata	+					
odora					+	

* HC - Hydrocarbon, ** Present Study

Table 16 (cont.)

Species	Carvacrol type	Thymol type	Monoterpenic alcohol type	Monoterpenic aldehyde type	Monoterpenic ketone type	Monote-rpenic-HC* type
parnassica var.*macro-phylla*					+	
parnassica ssp.*sipylea*	+					
parvifolia					+	
pseudosimensis					+	
*punctata***				+		
subspicata	+					
salzmanni			+			
spicigera		+				
thymbra	+	+				
taurica	+	+				
visiani		+				
vulgaris			+			

* HC - Hydrocarbon
** Present study

3. SUMMARY

Chemical investigations on the essential oils of *S. abyssinica* and *S. punctata* were conducted.

Satureja abyssinica

The plant has a pleasant fragrance and is used for different purposes [16]. However, no phytochemical study on *S. abyssinica* has been reported so, because of the local uses of the plant, we decided to analyse its essential oil.

Several works [18-29] done at the genus level indicate that pulegone [19, 24, 26] and carvacrol [21, 22, 25, 27, 28] are the most common major components of oils of *Satureja* species.

In the oil of *S. abyssinica*, pulegone is found to be the major component (69-79%).

Four components constituting 72-80% of the oil were isolated and identified.

Satureja punctata

No report has appeared in the literature regarding the oil of this plant. Chemical investigation on *S. punctata* revealed citral a and b to be the major components of the oil. On the basis of this, we propose to place *S. punctata* into a new grouping called "Monoterpenic aldehyde type" since the major component is a monoterpene aldehyde.

Ten components comprising 87-90% of the oil were isolated and identified. Citral a is reported as a major component for the first time. To the best of our knowledge, 3,7,10(15)- germacratriene is a novel natural product.

4. EXPERIMENTAL

Instruments

^{1}H NMR : Joel Fx 90Q (90 MHz), δ values are given in ppm

^{13}C NMR :Joel Fx 90Q (22.5 MHz)

GC : Varian 3700 model, fused silica capillary column coated with SE-54,

Column temperature : 60°C to 260°C at 18°C/min

FID temperature : 260°C

Injector block : 210°C

IR : Perkin Elmer 727 B

Optical rotation : Perkin Elmer Model 241 Polarimeter

Refractive index : Abbe's refractometer

Specific gravity : pycnometer, all measurements were conducted at 20°C.

Chromatography

Analytical TLC : Silica gel 60 F_{254} (Merck), 0.20 mm precoated plate.

Preparative TLC : Silica gel 60 $F_{254+366}$ (Merck), coated on glass plates.

Column chromatography : Silica gel 60 (Merck), 0.063-0.200 mm

Plant material

S. abyssinica and *S. punctata* samples were collected from different areas in Ethiopia.

Sample A-a : A sample of *S. abyssinica* collected from Shoa region, Adadi Mariam, 55 km South of Addis Ababa (Alt.2100 m) on Oct. 13, 1994; voucher no. Sebsebe D. and Ermias D. 4136.

Sample G-a : A sample of *S. abyssinica* collected from Shoa region, Guder, 250 km west of Addis Ababa (Alt. 2300 m) on Aug.12, 1994; voucher no. Melaku W., Gizachew A. and Solomon T. 81.

Sample DM-a : A sample of *S. abyssinica* collected from Dila to Moyale road (101 km from Dila, Alt. 1350 m) on Nov. 22, 1994; voucher no. Sebsebe D. and Ermias D. 4194.

Sample E-p : A sample of *S. punctata* collected from Addis Ababa, Entoto area, Higher 11 Kebele 04 (Alt. 2600m) on Nov. 8, 1994; voucher no. Melaku W. and Solomon T. 91.

Sample G-p : A sample of *S. punctata* collected from Shoa region, Guder (Alt. 2300m) on Aug. 12, 1994; voucher no. Melaku W., Gizachew A. and Solomon T. 79.

Sample M-p : A sample of *S. punctata* collected from Shoa region, Menagesha Awraja, Menagesha State Forest, 52 km West of Addis Ababa (Alt. 2890 m) on July 22, 1994; voucher no. Melaku W. and Solomon T. 76.

All voucher specimens have been authenticated by Dr Sebsebe Demissew and deposited at the National Herbarium (ETH.), Department of Biology, Addis Ababa University.

Isolation and Characterization

Distillation

The essential oil samples (A-a, G-a, DM-a, E-p, G-p, M-p) were obtained by hydrodistillation of the plants.

Isolation

Essential oil from *S. abyssinica*. 1.0 Gm of the oil was applied an a silica gel column and eluted successively with hexane and ethyl acetate to obtain hydrocarbon and oxygenated fractions, respectively. The oxygenated fraction was further fractionated on a silica gel column with 10% ethyl acetate in petroleum ether. A total of 46 fractions each of ca 10 ml were collected. Fractions 15-17 were further fractionated on a silica

gel column with 5% ethyl acetate in petroleum ether. About 14 fractions each of *ca* 3 ml were collected. Fractions 8-14 afforded pulegone (**1**).

Fractions 26-29 from the first CC were applied on a chromatotron and eluted with 10% ethyl acetate in petroleum ether. Eleven fractions each of *ca* 3 ml were collected and fractions 6-11 gave piperitenone (**2**).

Essential oil from *S. punctata*. 2.5 Gm of the oil was applied on a silica gel column and eluted successively with hexane and ethyl acetate to obtain hydrocarbon and oxygenated fractions, respectively. Column chromatography on the oxygenated fractions was done with 10% ethyl acetate in petroleum ether. A total of 91 fractions each of *ca* 3 ml were collected. Fractions 19-26 were applied on a chromatotron and eluted with chloroform. Eight fractions each of *ca* 2 ml were collected. Fractions 1-2 gave citral a (**4**).

Fractions 3-10 from the CC were further fractionated on a silica gel column with 8% diethyl ether in petroleum ether. About 60 fractions each of *ca* 5 ml were collected. Fractions 13-16 after purification by preparative TLC, afforded citral b (**3**) and caryophyllene oxide (**9**). Fractions 3-5 of the 60 fractions after purification by CC with 8% diethyl ether in petroleum ether, gave geranyl acetate (**5**).

Fractions 50-71 from the CC were further fractionated on a silica gel column with 10% diethyl ether in petroleum ether. About 60 fractions each of *ca* 5 ml were collected. Fractions 45-49 of these collections were applied on PTLC using chloroform and afforded α-bisabolol (**10**).

Fractions 80-91 from the first CC was further fractionated on a silica gel column with 10% diethyl ether in pet.ether. About 60 fractions *ca* 5 ml were collected. Fractions 37-60 of these collections were applied on a PTLC using 10% diethyl ether in petroleum ether. Two components were obtained. One of the components was further purified by PTLC using the same solvent and afforded nerolidol (**8**).

The hydrocarbon fraction was applied to a PTLC using cyclohexane as a solvent. About five components were obtained. Component 2 and 3 were further purified by PTLC using the same solvent and gave ß-caryophyllene (**6**) and 3,7,10(15)-germacratriene (**7a**), respectively.

Pulegone (1)

Colourless oily liquid; RT 5.75 min; IR ν_{max} cm^{-1} : 3748, 3358, 2954, 2924, 2871, 2365, 1682, 1618, 1455, 1373, 1286, 1209, 1131, 1096, 1029, 876; ^1H NMR (90 MHz, $CDCl_3$); δ 0.99 (3H, d, J=6.07 Hz, 7-H), 1.75 (3H, s, 9-H), 1.94 (3H, s, 10-H), 1.50-2.90 (3-H, 4-H, 5-H, 6-H): ^{13}C NMR (22.5 MHz, $CDCl_3$) : see Table 8.

Piperitenone (2)

RT 7.20 min; ^1H NMR (90 MHz, $CDCl_3$) ; δ 1.83 (3H, s, 10-H), 1.90 (3H, s, 9-H), 2.08 (3H, s, 7-H), 2.26 (2H, 5-H), 2.66 (2H, 6-H), 5.85 (1H, brs, 2-H)

Citral b (3)

Light yellow oily liquid; RT 5.56 min; IR $\nu_{max}cm^{-1}$: 3332, 2923, 2856, 2362, 1674, 1632, 1443, 1379, 1193, 1121, 844 ; ^1H NMR (90 MHz, $CDCl_3$) : δ 1.60 (3H, s, 8-H) 1.69 3H, s, 9-H), 1.98 (3H, s, 3-CH_3) 2.10-2.80 (4-H, 5-H), 5.11 (1H, br, 6-H), 5.85 (1H, d, J=8.60 Hz, 2-H), 9.89 (1H, d, J=8.60 Hz, 1-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) : see Table 9.

Citral a (4)

Light yellow oily liquid; RT 5.93 min; IR $\nu_{max}cm^{-1}$: 3854,3747,3330,2968, 2924, 2958, 2362, 1674, 1445, 1381, 1193, 1123, 1046, 847; ^1H NMR (90 MHz, $CDCl_3$) : δ 1.60 (3H, s, 8-H), 1.69 (3H, s, 9-H), 2.18 (3H, s, 3-CH_3), 2.10-2.40 (4-H, 5-H), 5.03 (1H, br, 6-H), 5.85 (1H, d, J=8.25 Hz, 2-H), 9.96 (1H, d, J=8.25 Hz, 1-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) : see Table 10.

Geranyl acetate (5)

RT 6.59 min; ^{1}H NMR (90 MHz, $CDCl_3$); δ 1.65 (3H, s, 10-H), 1.75 (3H, s,11-H), 2.01 (3H, s, 6-Me), 2.01-2.02 (6-H, 7-H), 2.03 (3H, s,1-H), 4.52 (2H, d, J=9 Hz, 3-H), 5.06 (1H, br, 8-H), 5.35 (1H, t, J=9 Hz, 4-H).

β-Caryophyllene (6)

Colourless oily liquid; RT 6.70 min; IR $\nu_{max}cm^{-1}$: 3421, 3068, 2927, 2362, 1634, 1453, 1374, 1276, 1181, 805; ^{1}H NMR (90 MHz, $CDCl_3$); δ 0.92 (3H, s, 13-H), 1.19 (3H, s, 12-H), 1.41 (2H, br, H-2), 1.57 (3H, s, 15-H), 1.70 (2H, m,3-H), 2.32 (2H, m, 6-H), 4.80 (1H, br, 14-H), 4.91 (1H, br, 14'-H), 5.21 (1H, br, 5-H); ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 11.

3,7,10(15)-Germacratriene (7a)

Colourless solid; RT 7.47 min; ^{1}H NMR (300 MHz, $CDCl_3$); δ 0.82 (3H, d, J=6.12 Hz, H-12), 0.89 (3H, d, J=6.12 Hz, H-13), 1.20-2.00 (H-1, H-2, H-5, H-6, H-11), 1.60 (3H, s, H-14), 4.75 (1H, d, J=14.1 Hz H-15), 4.79 (1H, d, J=14.1 Hz, H-15'), 5.15 (1H, t, J=6.2 Hz, H-8), 5.40 (1H, brs, H-3); ^{13}C NMR (22.5 MHz, $CDCl_3$) : see Table 13. MS : m/z (base peak 134), 204, 161, 134, 119, 109, 93, 81.

Nerolidol (8)

Colourless oily liquid; RT 7.97 min; ^{1}H NMR (300 MHz, $CDCl_3$); δ 1.20 (3H, s, 3-Me), 1.51 (6H, s, 11-Me, 12-H), 1.68 (3H, s, 7-Me), 1.20-2.40 (4-H, 5-H, 8-H, 9-H), 4.95 (2H, d, J=2.90 Hz, 1-H), 5.09 (1H, S, -O<u>H</u>), 5.09 (1H, br, 10-H), 5.25 (1H, br, 6-H), 5.91 (1H, br, 2-H) ; ^{13}C NMR (75 MHz, $CDCl_3$): see Table 14.

Caryophyllene oxide (9)

RT 8.27 min; IR $\nu_{max}cm^{-1}$: 3420, 2930, 2861, 2363, 1132, 1632, 1457, 1382, 1279, 1072, 891, 862; ^1H NMR (300 MHz, $CDCl_3$) : δ 0.94 (3H, s, 13-H), 1.16 (3H, s, 15-H), 1.21 (3H, s, 12-H), 1.35 (2H, m, H-6), 1.65 (2H, m, H-2), 1.73 (2H, m, 10-H), 2.07 (1H, m, H-1), 2.12 (2H, m, 3-H), 2.90 (1H, dd, J=2, 11 Hz, H-5), 4.86 (1H, d, J=1 Hz, H-14), 4.97 (1H, d, J=1 Hz, H-14').

α-Bisabolol (10)

RT 8.94 min; ^1H NMR (300 MHz, $CDCl_3$) : δ 1.10 (3H, s, 7-CH_3), 1.62 (3H, s, 11-CH_3), 1.65 (3H, s, 12-H), 1.68 (3H, s, 3-CH_3), 2.01 (1H, s, 7-O<u>H</u>), 5.13 (1H, t, J=6.43 Hz, 10-H), 5.38 (1H, br, 2-H); ^{13}C NMR (75 MHz, $CDCl_3$) : see Table 15.

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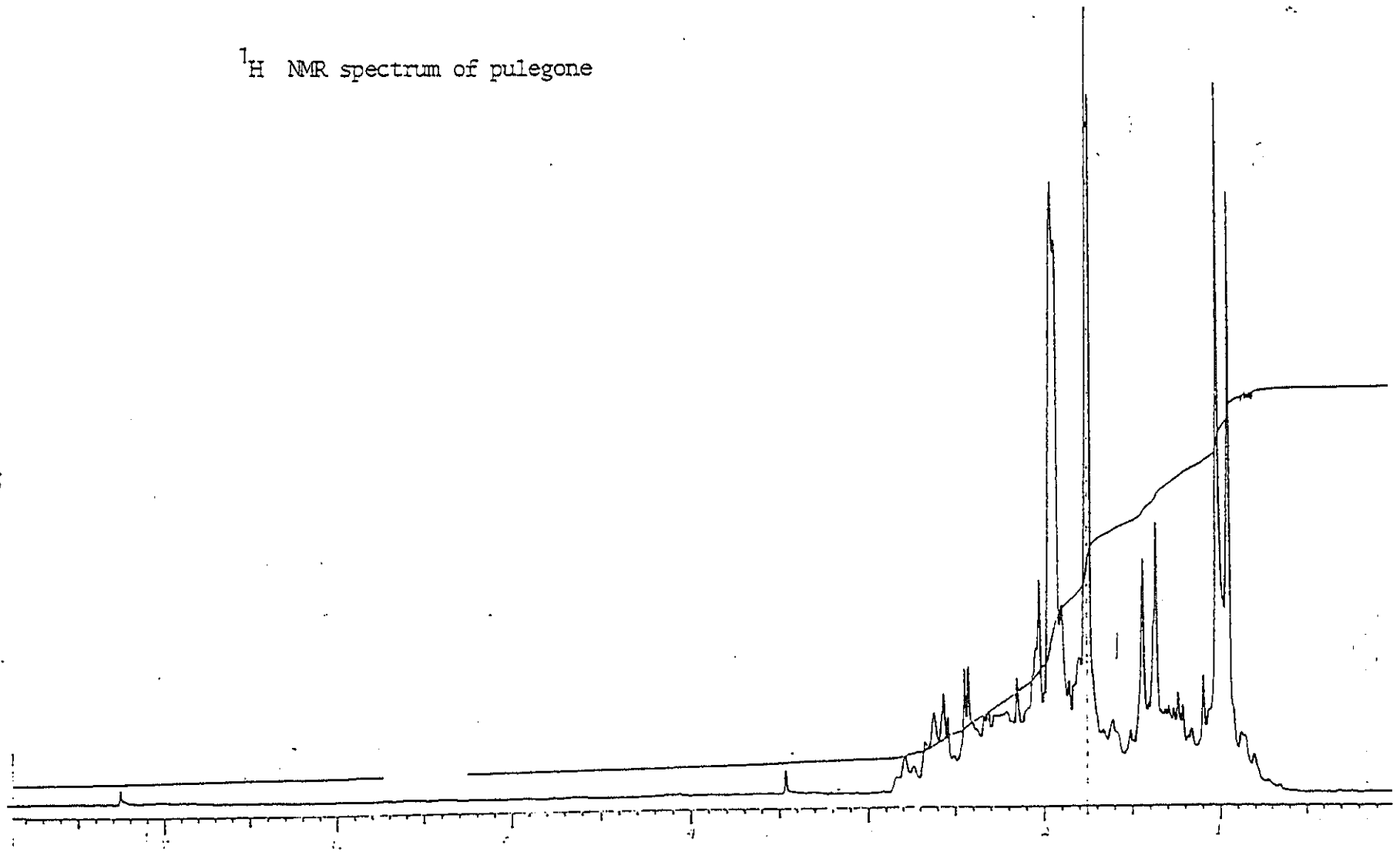
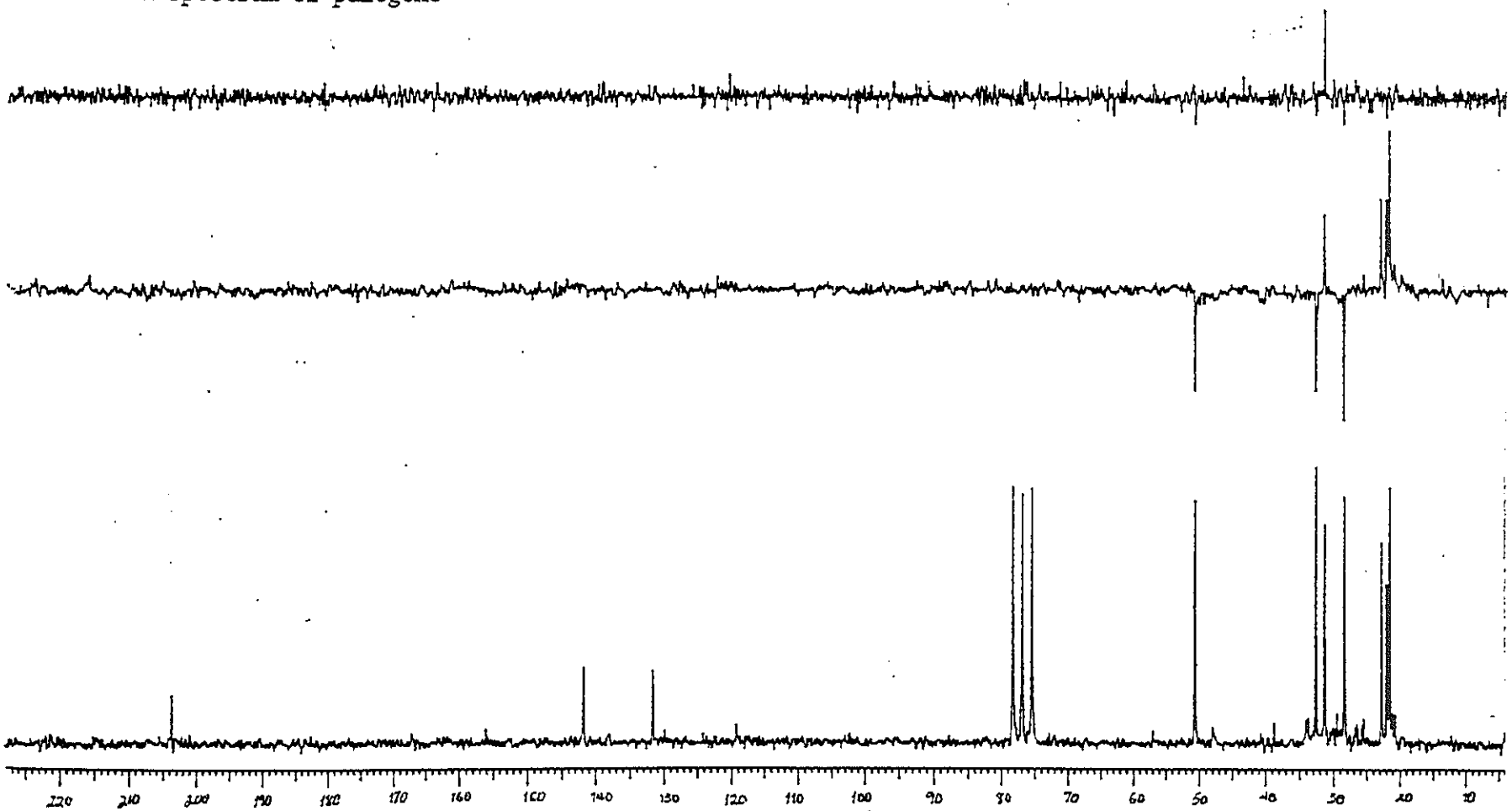
APPENDIX

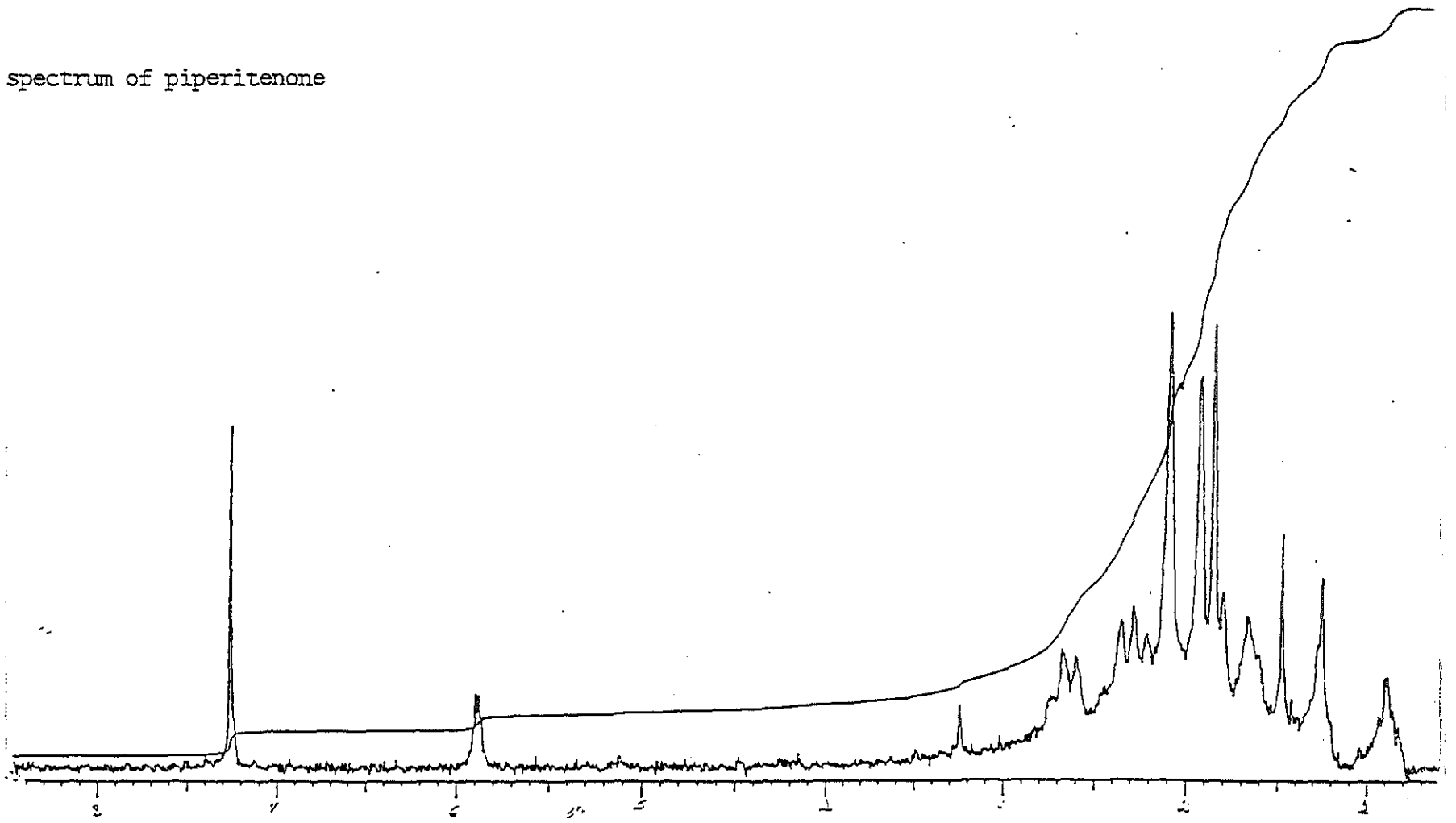
^1H NMR spectrum of pulegone

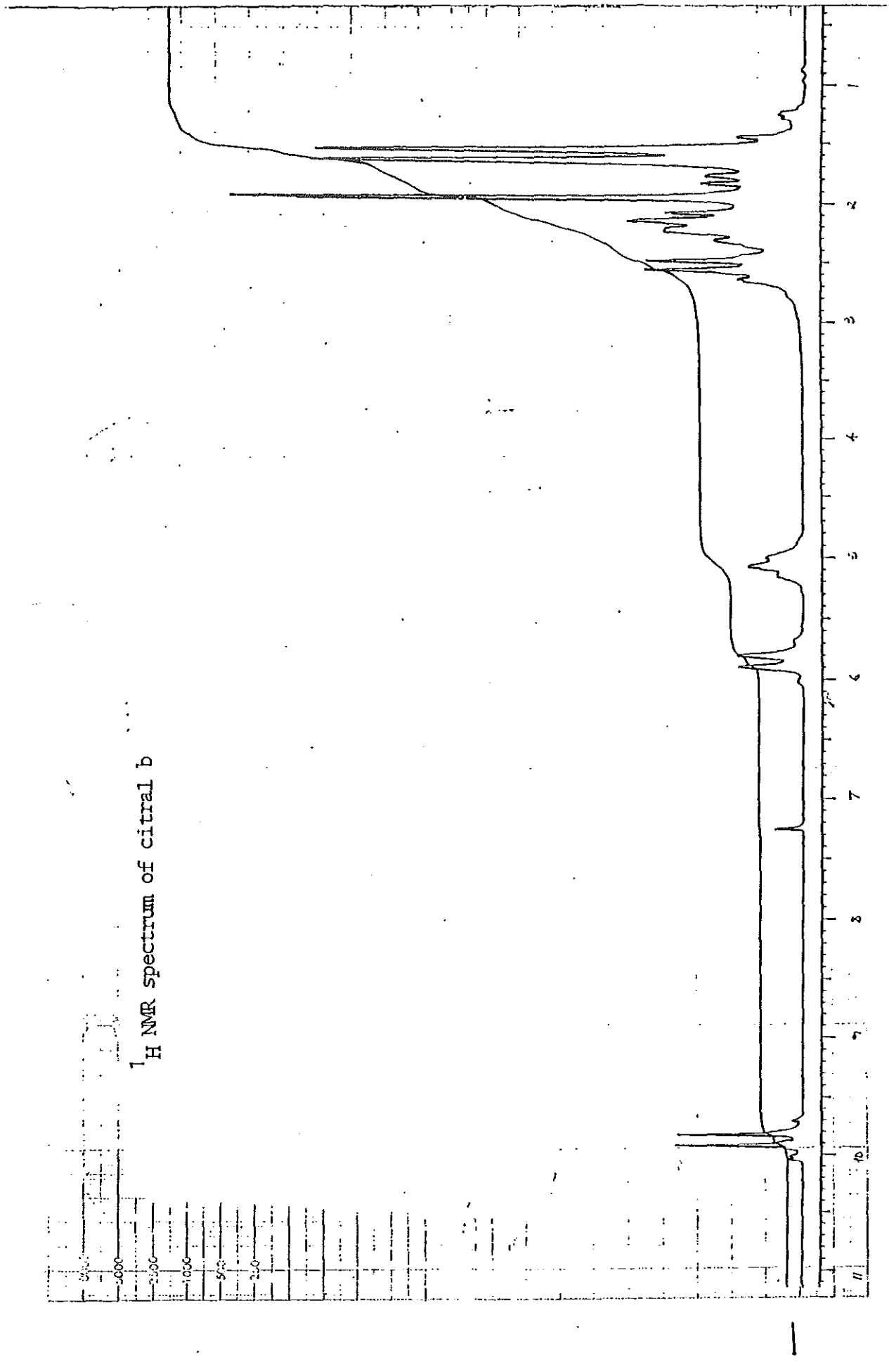


^{13}C NMR spectrum of pulegone



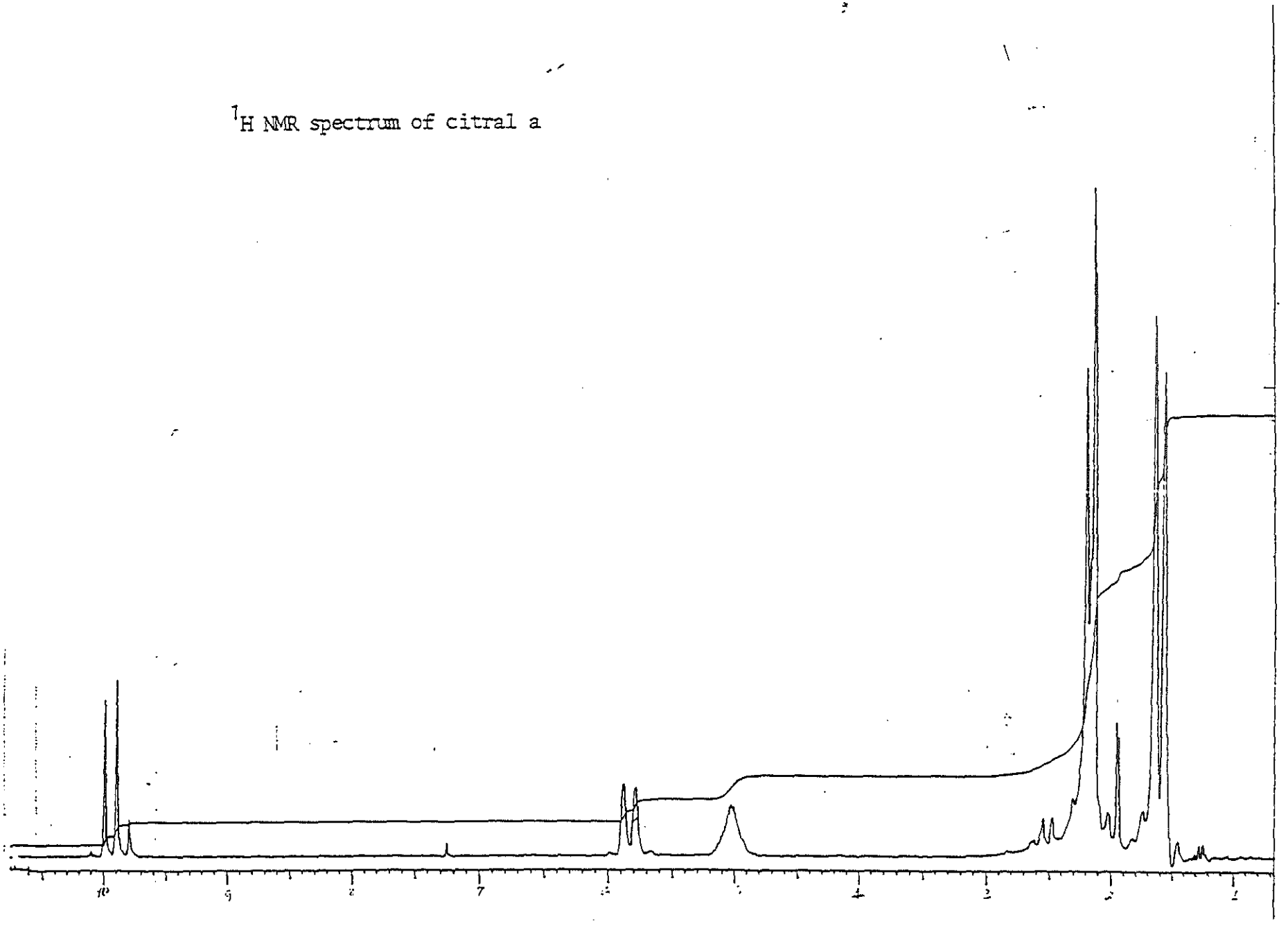
^{1}H NMR spectrum of piperitenone



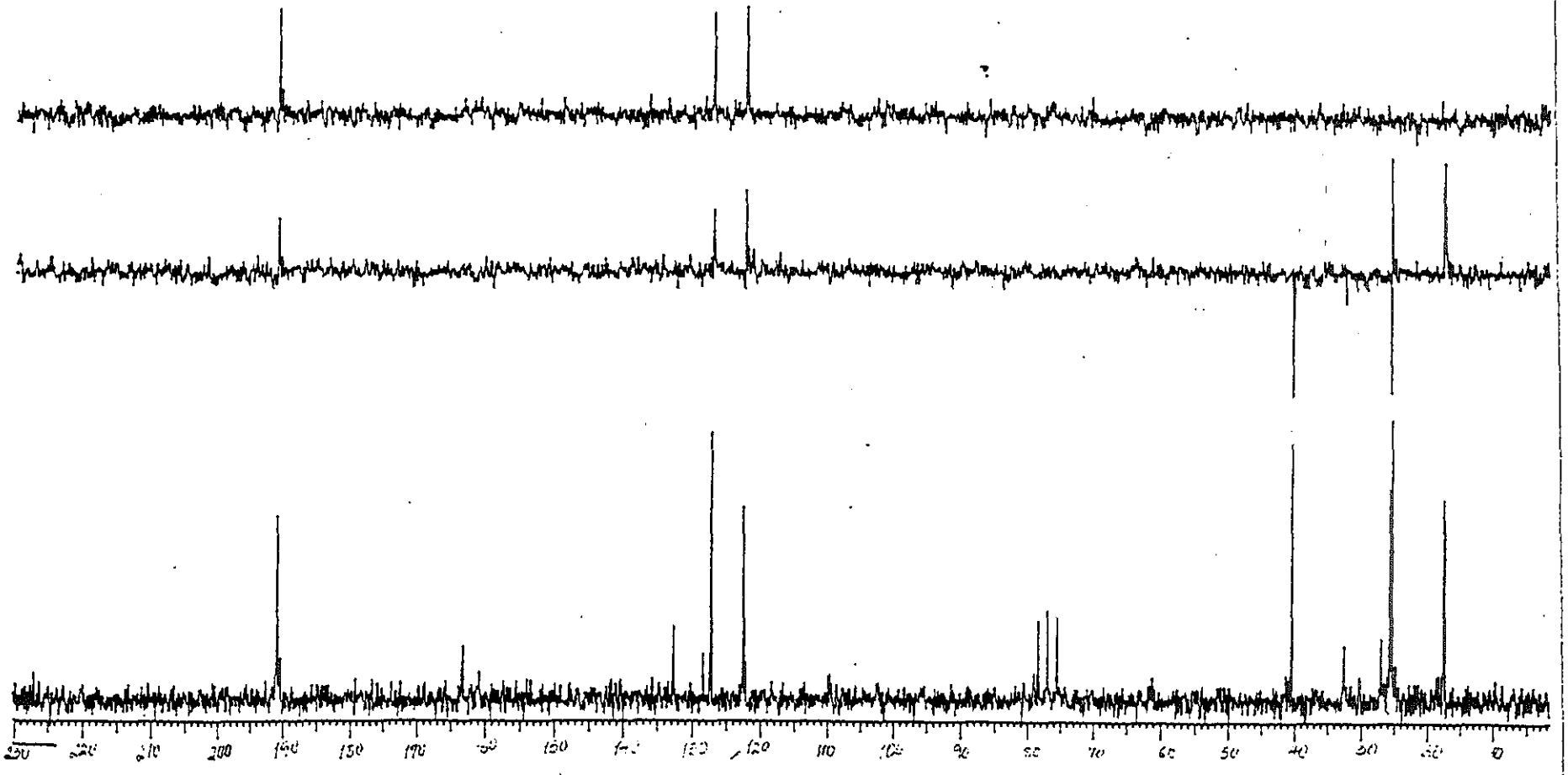


^1_H NMR spectrum of citral b

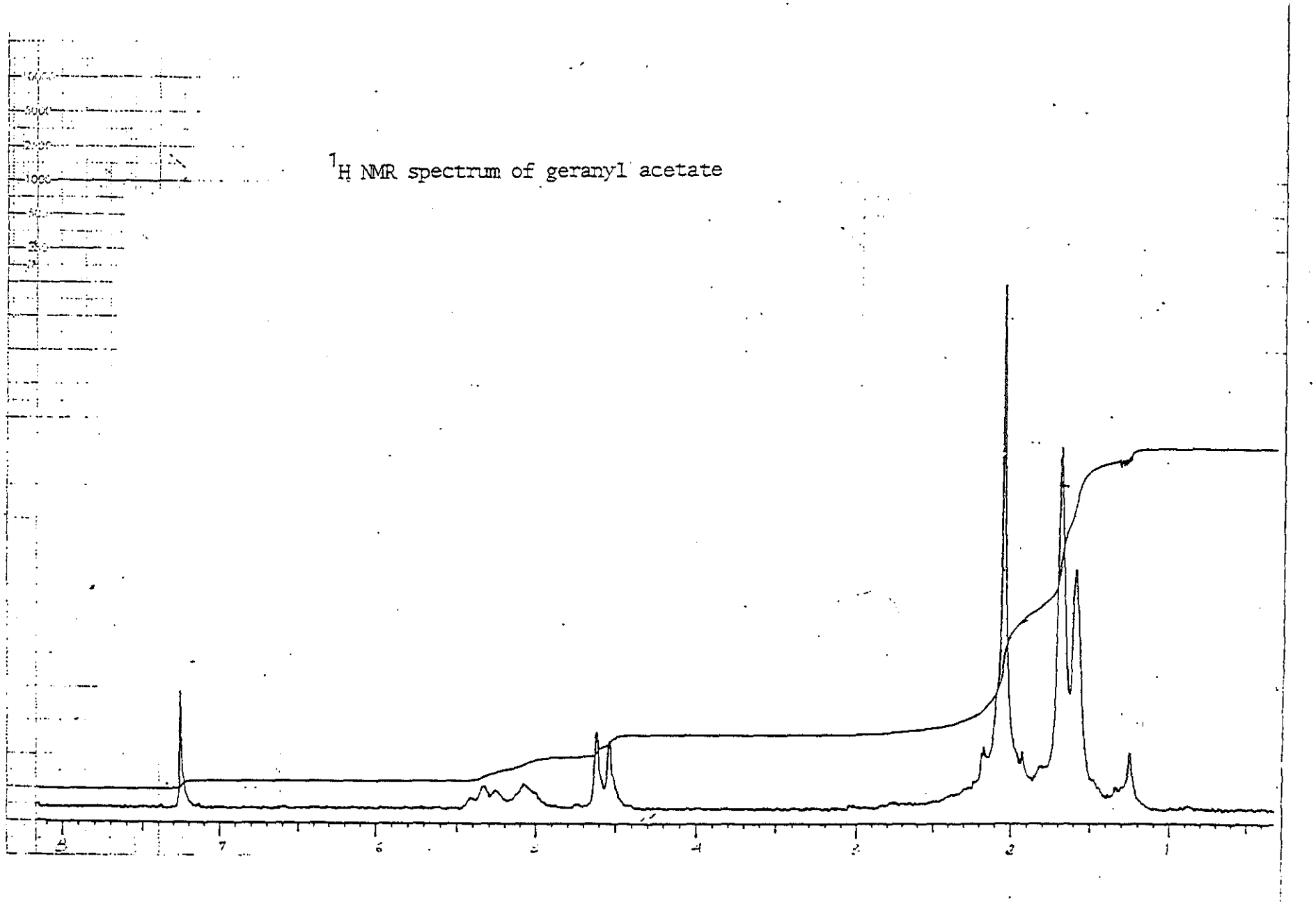
^{1}H NMR spectrum of citral a



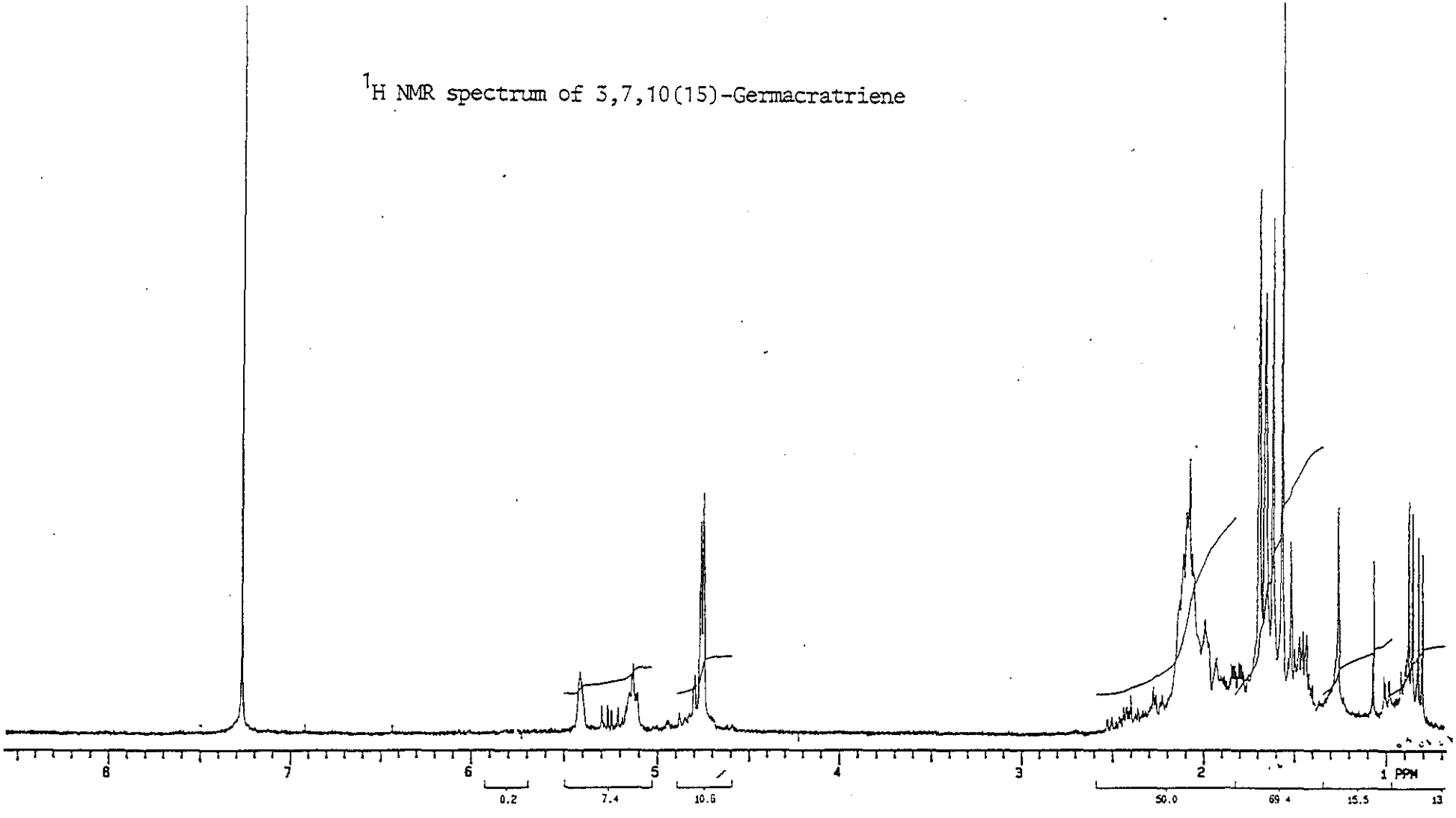
^{13}C NMR spectrum of citral a



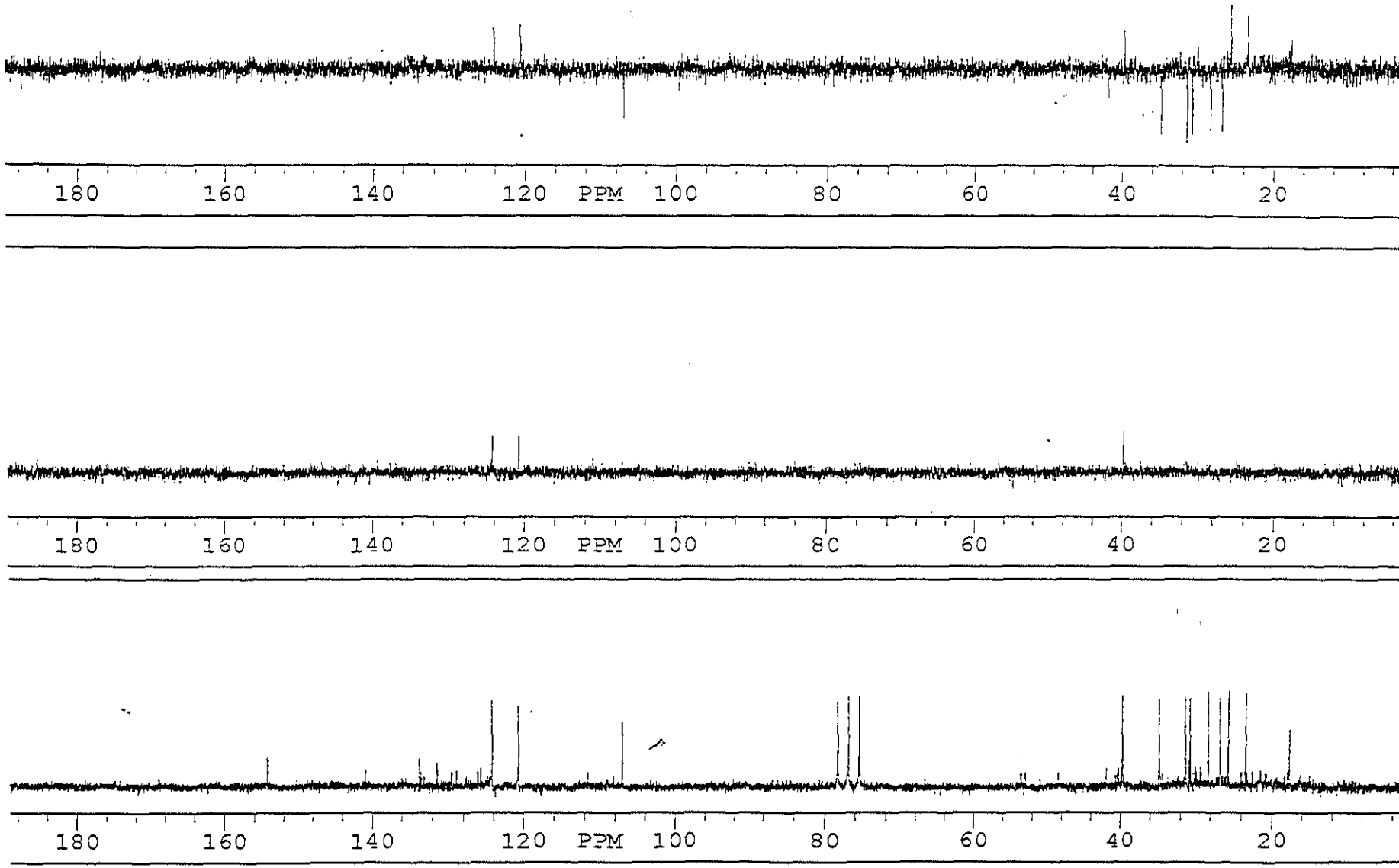
^1H NMR spectrum of geranyl acetate



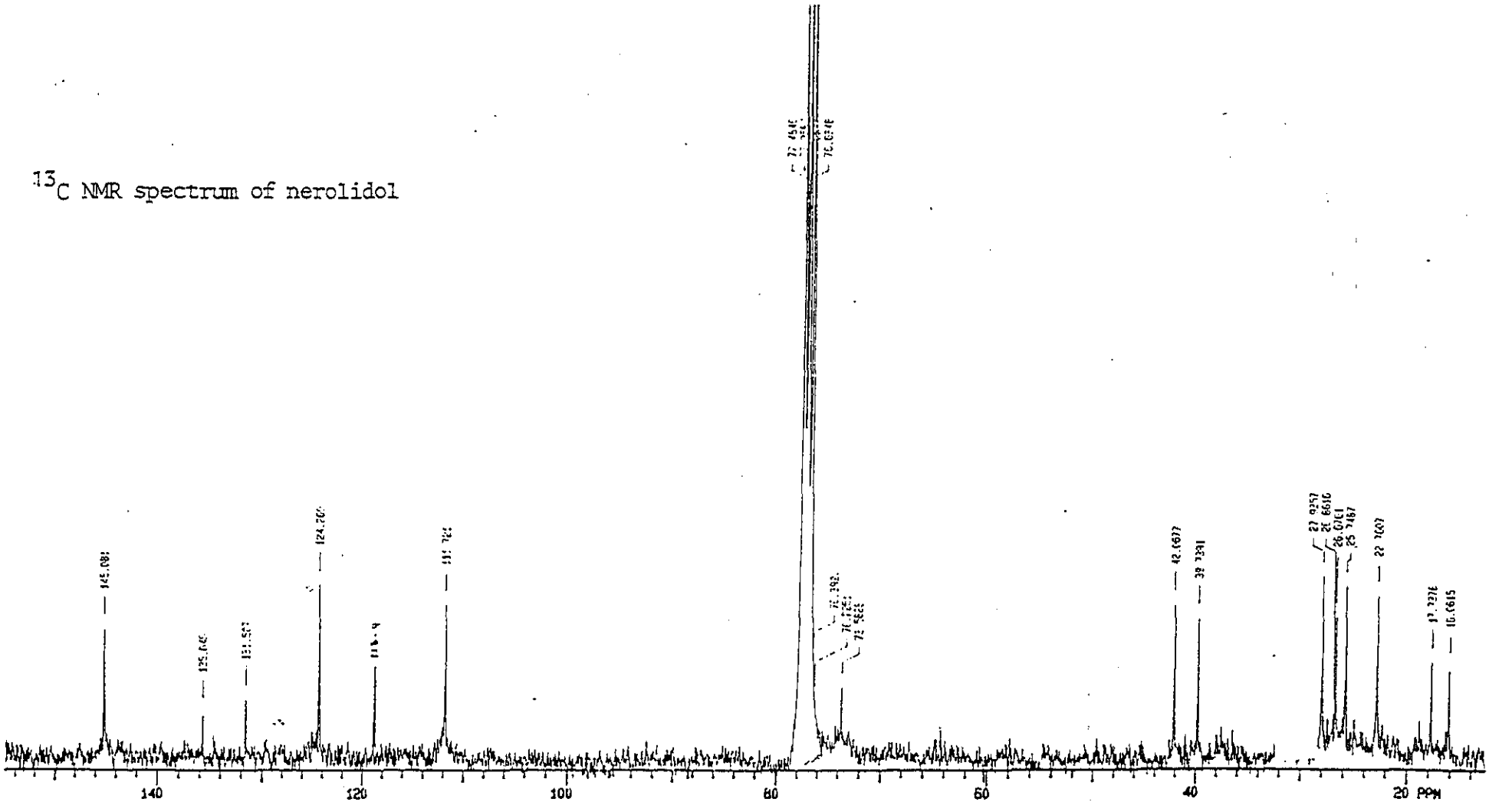
^{1}H NMR spectrum of 3,7,10(15)-Germacratriene



^{13}C NMR spectrum of 3,7,10(15)-Germacratriene



^{13}C NMR spectrum of nerolidol



DECLARATION

I the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been duly acknowledged.

Name : Solomon Teklu

Signature : 

Date : June, 1995

Place of submission : Chemistry Department, Addis Ababa University