CHEMICAL INVESTIGATION ON THE ESSENTIAL OILS OF ENDEMIC WILD AND CULTIVATED *LIPPIA ADOENSIS*: A COMPARATIVE STUDY.

A Thesis submitted to the School of Graduate Studies
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In Partial Fulfilment of the Requirements for the Degree of
Masters of Science in Chemistry

By
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Addis Ababa
June, 1992

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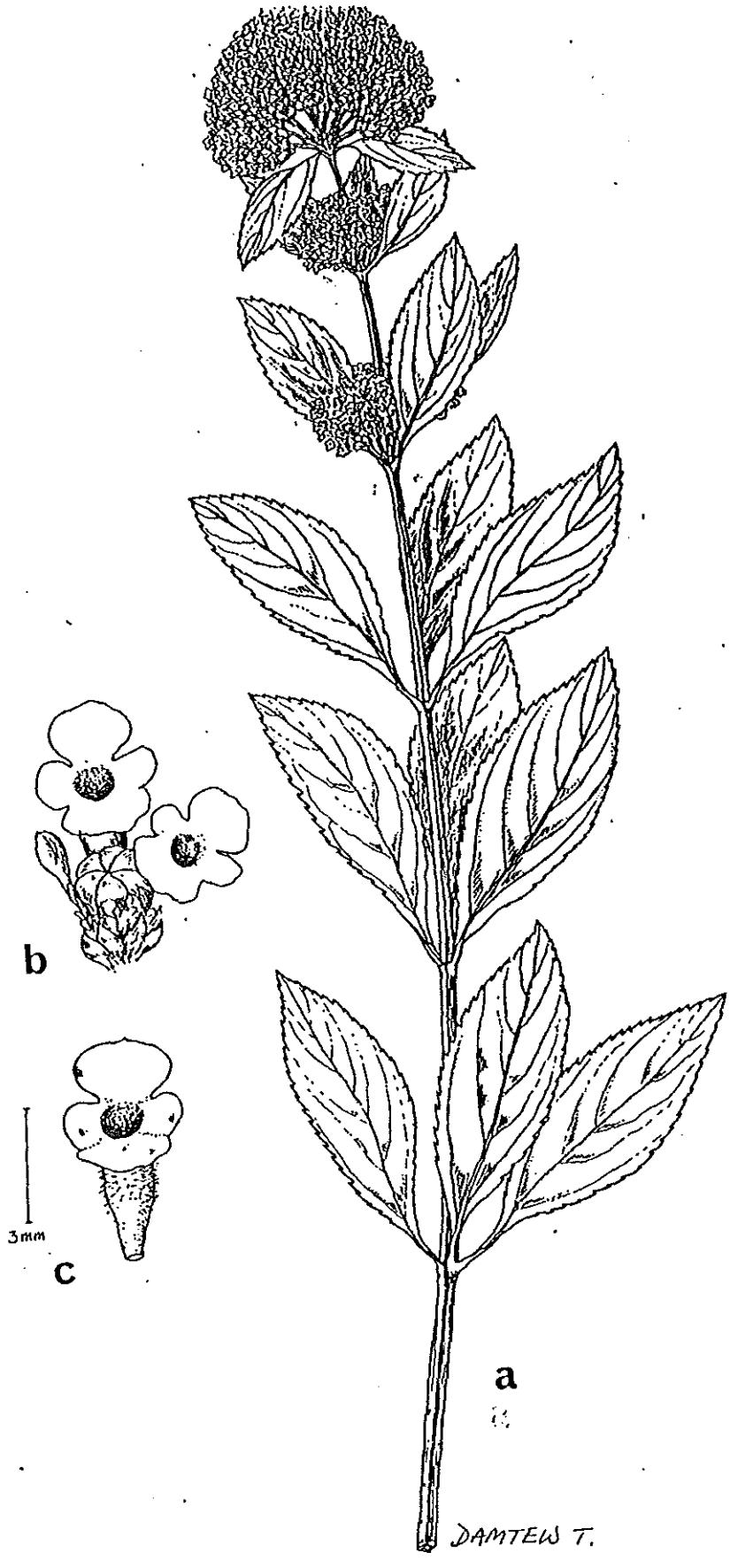

Lippia adoensis

a. Branch with flowers b. Enlarged inflorescence c. Enlarged flower

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CHEMICAL INVESTIGATION ON THE ESSENTIAL OILS OF ENDEMIC WILD AND CULTIVATED LIPPIA ADOENSIS: A COMPARATIVE STUDY

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ABSTRACT

study on the essential oil of endemic *L. adoensis* Hochst. ex. Walp. was undertaken. The :search work was conducted on samples from four areas where the ethnic groups claim a ifference in the flavour of the wild and cultivated *L. adoensis*. The chemical investigation ıdicated that the essential oils obtained from the wild and cultivated *L. adoensis* are markedly ifferent in their physical characteristics and chemical composition. The essential oils from these vo types were differentiated from each other by the presence of l-linalool as a major component ı the cultivated and its absence in the wild; and by the presence of d-limonene, perillaldehyde, ıiperitenone, and citral a in the wild and their absence in the cultivated *L. adoensis*. The oils listilled from cultivated *L. adoensis* show a laevo rotation while those from the wild are dextro otatory. Morphological differences of the leaves were also observed. There are no published eports on the morphological or chemical differences between the wild and cultivated *L. adoensis* ›rior to this work.

Fourteen components comprising 88.1 - 94.8% of the oil from the cultivated, and 16 components :onstituting 68.4 - 86.5% of the oil from the wild were identified. These include ipsdienone, l-inalool, germacrene D, d-limonene, d-perillaldehyde, piperitenone, citral a and b, ocimene, α-:opaene, α- and ß-caryophyllene, α-farnesene, α- and ß-cadinene. To the best of our knowledge, this is the first time that ipsdienone is reported from a natural source. All the other compounds are known. However, piperitenone, perillaldehyde, germacrene D, citral a and b, have not been reported before in *L. adoensis*.

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 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 or exceptionally in heartwoods [1]. The function of the essential oils in plants is not satisfactorily explained, although they sometimes serve as attractants, repellents, or protectants [2].

Essential oils have wide and varied industrial applications. They are used in the manufacture of perfumes, cosmetics, and toilet soaps [3]; as flavouring materials in candy, chewing gum, ice cream, and for flavouring alcoholic as well as nonalcoholic beverages [4]. Still others have therapeutic or bactericidal properties and are valuable in medicine and dentistry [5].

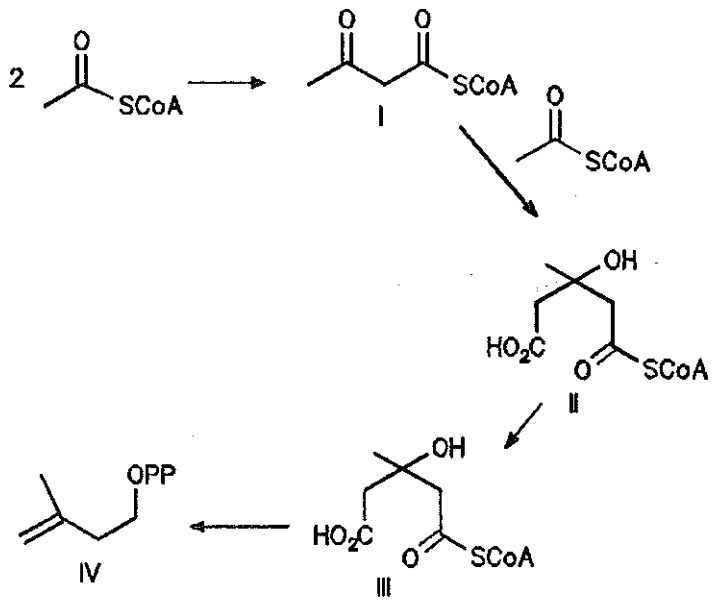
1.1.1 Composition of essential oils

The main constituents of the essential oils are the terpene hydrocarbons and related oxygenated terpenoid derivatives [2]. The aromatic phenylpropanoid compounds, which consist mainly of substituted propenyl phenols are the other class of compounds found in essential oils. Of the terpenes, the monoterpenes and sesquiterpenes are the most abundant components of essential oils.

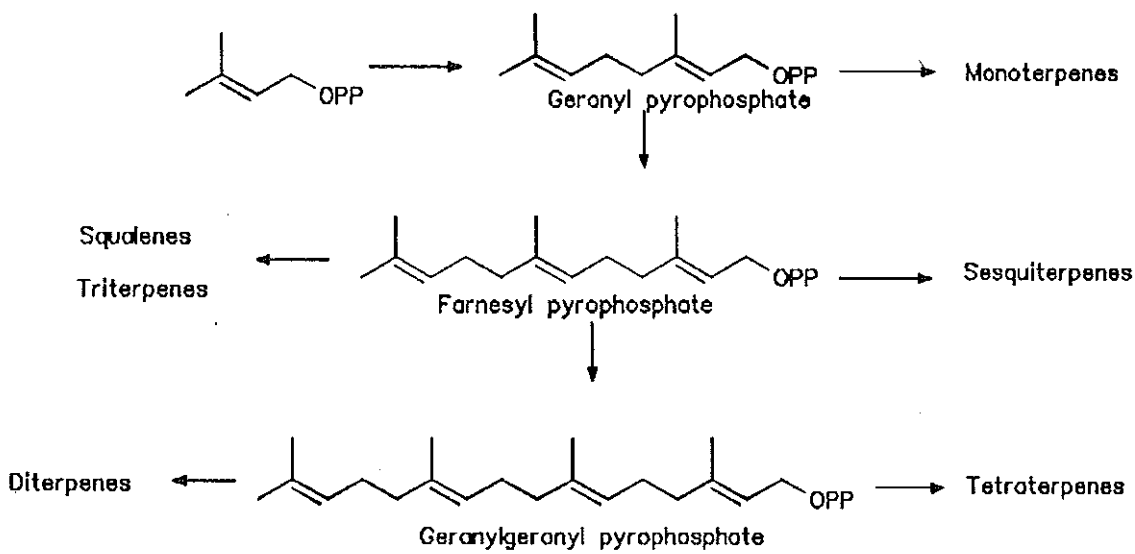
1.1.2 Biogenesis of terpenoids

Terpenoids are products of secondary metabolism. 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 (IV), arises from mevalonic acid (III) *via* hydroxymethylglutarate (II), (Scheme 1) [6]. The metabolic pathway is believed to start with the condensation of two molecules of acetic acid to form aceto

acetyl coenzyme A (I). Isopentenyl pyrophosphate is then readily converted to terpenes by polymerization in a variety of ways (Scheme 2) [7].



Scheme 1: Biogenesis of isopentenyl pyrophophate

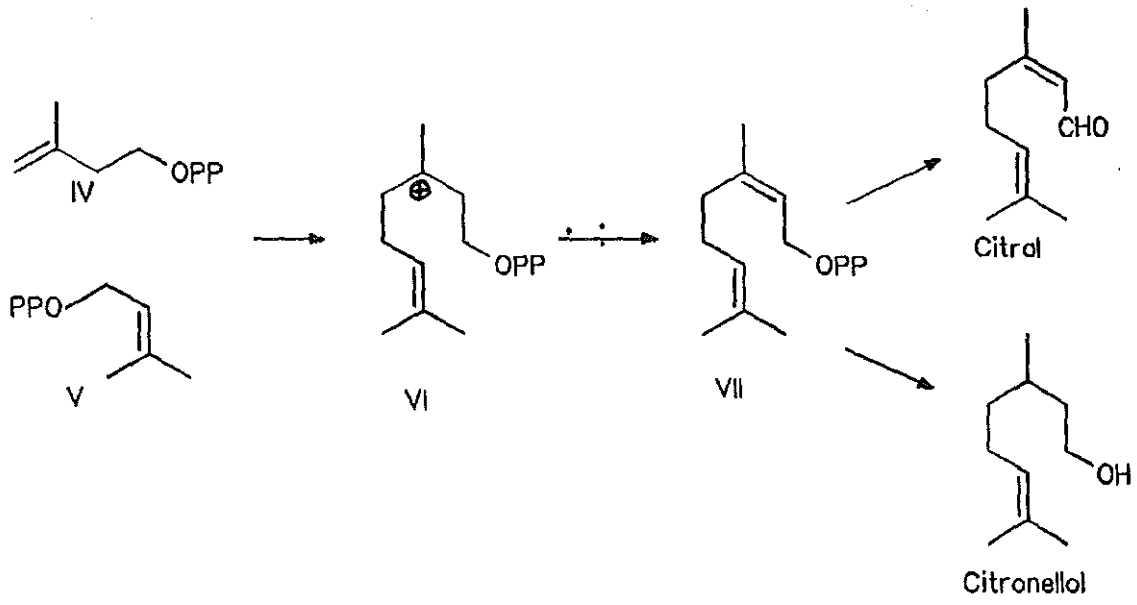


Scheme 2: Biogenesis of terpenoids

1.1.2.1 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 (IV) and dimethylallyl pyrophosphate (V) to give geranyl pyrophosphate (VII) *via* the cation (VI), (Scheme 3) [2].

Monoterpenes are widely used in the flavour and perfume industries because of their attractive odours, low molecular weights, and high volatilities.

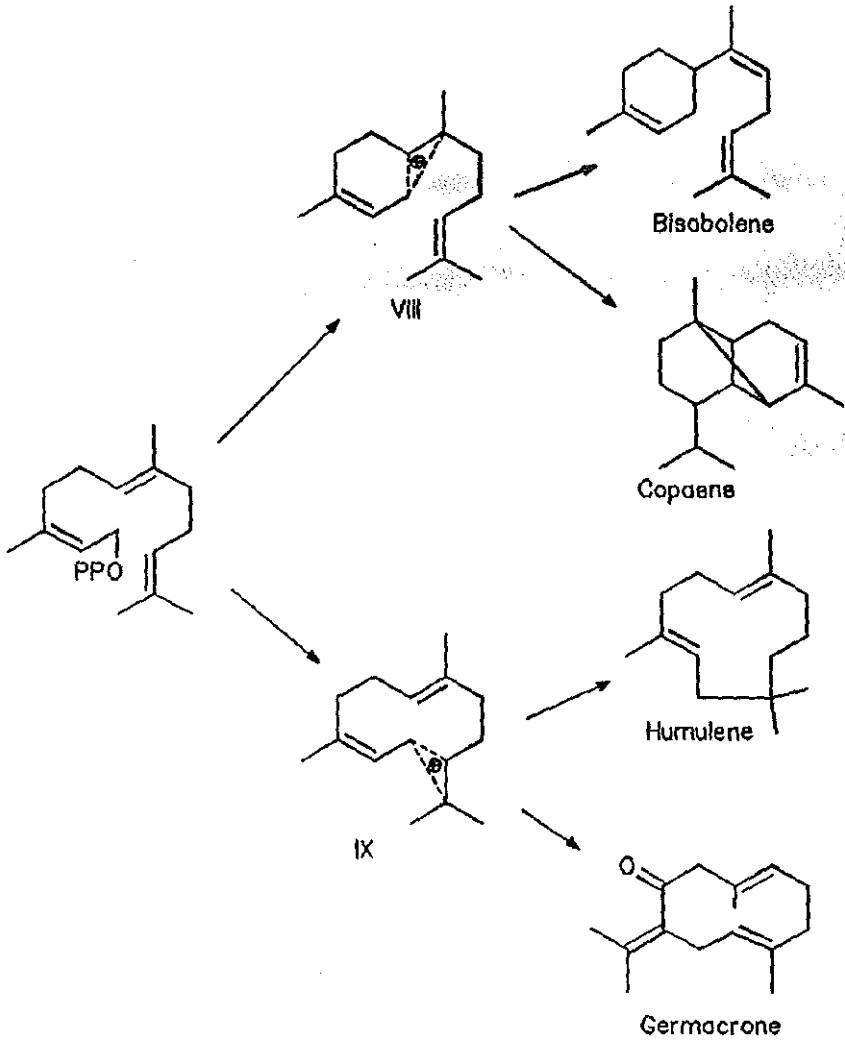


Scheme 3: Biogenesis of monoterpenes

1.1.2.2 Sesquiterpenes

These are C_{15} hydrocarbons or their oxygenated analogs. They arise from the cyclization of farnesyl pyrophosphate and subsequent rearrangements of the resulting carbonium ions (VIII,

IX) (Scheme 4) [2, 8].



Scheme 4: Biogenesis of sesquiterpenes

1.2 Domestication of wild plants

Domestication is the process whereby plants are changed genetically by human selection under cultivation, usually in such a way as to render them less fit for survival in the wild [9].

From the time a plant is taken into cultivation, man consciously or unconsciously, selects for characters which are useful to him, bringing about genetic, physiological, and structural changes [10]. These changes include:

1. spread to greater diversity of environments and a wider geographical range, some of them unsuited to the wild plant;
2. the crop may come to have a different ecological preference;
3. absence of shattering or scattering of seeds and sometimes a complete loss of dispersal mechanisms;
4. increase in size of fruits and seeds, often reducing dispersal efficiency;
5. improvement in palatability and chemical composition thus rendering them more likely to be eaten.

A fully domesticated plant or animal is completely dependent upon man for survival. Therefore domestication implies a change in ecological adaptation, and this is usually associated with morphological adaptation. The parts of the plant that show the greatest morphological alterations are the parts most valued by man. Speciation rarely occurs under domestication [11].

1.3 The genus *Lippia*

The genus *Lippia*, in the family Verbenaceae has about 200 species distributed in tropical Africa and America [12]. In Ethiopia, there are five *Lippia* species namely, *L. adoensis* Hochst. ex Walp., *L. javanica* (Burm. f.) Spreng., *L. grandifolia* Hochst. ex Walp., *L. dauensis* (Chiov.) Chiov. and *L. carviodora* Meikle [13].

The leaves of some *Lippia* species are used as tea or spice in Africa [14], Central and South America [15]. Others are used as remedies for abdominal complaints, cold and fever [16]. There are also some species which are credited with antifertility properties [17], while others are important in perfumery [15].

Chemical investigations on the essential oils of about 32 species of the genus are documented in the Chemical Abstracts (CA 1907 - 1991). Table 1 summarizes the compounds that have been identified from the essential oils of some *Lippia* species.

1.4 *Lippia adoensis*

L. adoensis is endemic to the afromontane region in Ethiopia. It is common in disturbed areas and forest margins at altitude of 1900 - 2650 m in Eritrea, Tigray, Gondar, Wello, Gojam, Shoa, Harerge, Arsi, Sidamo, Gamo Gofa, Keffa and Wellega Administrative Regions [13].

The wild *L. adoensis* known locally as *Kesse* is used for washing wooden and ceramic utensils to impart fresh and spicy fragrance. It also has some medicinal applications. The boiled leaves and flowers are taken against indigestion, flu, and headache [18].

The *L. adoensis* cultivated in the gardens of the Gurage and Oromo ethnic groups in Shoa Administrative Region is called *Kosseret*. The leaves of *Kosseret* are used for flavouring purposes and they are claimed to have superior flavour compared to the wild *L. adoensis* (*Kesse*). The dried leaves of cultivated *L. adoensis* (*Kosseret*) are important ingredients in the preparation of spiced butter giving it a characteristic sweet aroma and flavour. Spiced butter is an important cooking fat in the preparation of Ethiopian dishes, like *Doro Wat*, *Kitfo*, etc. Several spices are used in making flavoured (spiced) butter. Some of which are Ethiopian cardamom, basil, fenugreek, rue, garlic turmeric, white cumin, and black cumin. Although individuals have certain personalized ways of blending spices with butter, most communities generally have more or less identical recipes for preparing spiced butter. The Gurages are generally regarded as the makers of the most popular *Kitfo* - a dish prepared from minced, fresh, lean, raw meat served in just melted spiced butter. *Kitfo* may also be cooked rare or medium. The Gurage *Kitfo* is characterized by the unique flavour imparted to it mainly from *Kosseret*, used in the preparation of spiced butter.

Table 1: Constituents of the essential oils of Lippia species

Compound	L. adoensis	L. aff. aristata	L. aristata	L. aff. sidoises	L. alba	L. alnifolia	L. carvio dora	L. citriodora	L. dulcis	L. fissicalyx
α- Pinene	+	+	+	+				+	+	+
Camphene						+			+	+
ß- Pinene	+		+						+	+
Limonene		+					+	+	+	+
Myrcene				+	+				+	
γ- Terpinene	+				+	+				
Sabinene		+	+	+						
p- Cymene				+	+	+				
Camphor				+					+	
1,8- cineole	+			+	+			+		+
Menthone										+
Pulegone										+
Piperitone										+
Carvone							+			+
Linalool	+	+					+	+	+	+
Geranial								+		
Neral								+		
Carvacrol	+			+		+				
Thymol	+			+						
Eugenol						+				
O-Methyl-Thymol						+				
δ- Cadinene	+	+	+		+				+	
ß-Caryophyllene		+	+	+	+	+			+	
α- Copaene	+				+				+	
ß- Elemene		+	+		+					
α- Humulene		+	+		+	+				

Table 1: Continued

Compound	Lippia grandis	Lippia grata	Lippia graveolens	L. grisebachiana	L. integrifolia	L. javanica	L. multiflora	L. origanoids	L. ukambensis
α- Pinene		+		+	+		+		+
Camphene		+		+					+
ß- Pinene				+					+
Limonene		+		+	+		+		+
Myrcene				+			+		
γ- Terpinene		+					+	+	
p- Cymene	+	+	+	+		+		+	
Camphor					+				+
1,8- cineole			+	+	+				+
Pulegone				+					
Piperitone				+					
Linalool	+			+		+	+		
4- Thujanol									+
Carvacrol	+	+	+				+		
Thymol	+	+	+				+	+	
Eugenol				+					
O-Methyl-Thymol		+							
ß-Caryophyllene		+		+		+	+		
α- Copaene		+							

It is interesting to note that, while the casual observer is unable to tell the difference in the appearance of *Kesse* and *Kosseret*, the Gurage growers of *Kosseret* are capable of identifying *Kesse* adulterants in bundles of *Kosseret* that are brought to the market for sell.

Much of the previous work on essential oils from *L. adoensis* was carried out before 1940 [19-23]. P. Rovesti in 1972 [18, 24] and S.D. Elakovich & B.O. Oguntimein in 1987 [25] have analyzed the essential oil of *L. adoensis* from Ethiopia and Nigeria, respectively. They reported different substances as the major component of the essential oil.

There are no written reports concerning the chemical or morphological differences of the cultivated and wild *L. adoensis* prior to this work.

1.5 Objectives

The main objective of this research work was to study the chemical composition of the essential oils derived from endemic *L. adoensis*. The difference in flavour of the wild and cultivated types claimed by the ethnic groups and the absence of written reports on the chemical or morphological differences interested us to investigate the essential oils derived from the wild (*Kesse*) and cultivated (*Kosseret*) *L. adoensis* in order to find out if the differences are reflected in the essential oil content qualitatively.

2. RESULTS AND DISCUSSION

The flowers and leaves of wild and cultivated *L. adoensis* were subjected to hydrodistillation. The essential oils obtained **(A-1, A-2), (C-1, C-2), (E-1, E-2), (G-1, G-2)** from the cultivated, and **(B-1, B-2), (D-1, D-2), (F-1, F-2), (H-1, H-2)** from the wild (flowers and leaves, respectively) were subjected to further analyses as detailed below.

2.1 Yield and physical characteristics

The essential oil yield of the cultivated *L. adoensis* range from 0.25 - 0.93% and from 0.93 - 1.29% for the flowers and the leaves, respectively (Table 2). The wild *L. adoensis* yielded essential oils in the range of 0.53 - 1.28% for the flowers and 0.81 - 1.46% for the leaves (Table 3). The essential oil yields of the leaves were found to be higher than the flowers for both wild and cultivated *L. adoensis*.

Table 2: Yield and physical characteristics of essential oils from cultivated *L. adoensis*

Sample Code	Yield (%v/w)	Refractive index	Optical Rotation	Specific gravity
A-1	0.25	1.4695	-31.70	------
A-2	1.20	1.4680	-31.65	0.8740
C-1	0.35	1.4682	------	------
C-2	1.29	1.4665	------	------
E-1	0.80	1.4675	-29.30	------
E-2	0.93	1.4699	-34.96	0.8772
G-1	0.93	1.4663	------	------
G-2	0.98	1.4692	-32.30	0.8770

Table 3: Yield and physical characteristics of essential oils from wild *L. adoensis*

Sample code	Yield (% v/w)	Refractive index	Optical rotation	Specific gravity
B-1	0.53	1.4925	+40.20	0.9146
B-2	1.02	1.4950	+36.61	0.9166
D-1	1.28	1.4995	+34.10	------
D-2	1.46	1.5010	+32.16	0.9180
F-1	0.96	1.4987	+29.79	0.9111
F-2	1.28	1.5042	+27.40	0.9142
H-1	0.81	1.4895	+42.50	0.9169
H-2	0.87	1.4960	+41.50	0.9187

The refractive index and specific gravity values were found to be higher for the oils from the wild *L. adoensis* than those of the cultivated ones (Tables 2 & 3). The optical rotation values are positive for the oils from the wild and negative for those of the cultivated *L. adoensis*.

Measurements of physical characteristics like refractive index, specific gravity, and optical rotation have proved to be of great value in the essential oil industry because of their simplicity and rapidity in ascertaining the purity of the oil samples. For instance, the vetiver oils of wild and cultivated origin, which possess different and opposite rotations, are easily distinguished by optical rotation measurements [26]. Likewise, measurement of optical rotation would be a quick method for distinguishing the oils from the wild and cultivated *L. adoensis*.

2.2 Chemical composition

The essential oils obtained from the flowers and leaves of both wild and cultivated *L. adoensis* were subjected to gas chromatographic analyses. The oils from the cultivated were found to be different from the wild and the results are presented below.

2.2.1 Essential oils from the cultivated *L. adoensis*

The essential oils from the flowers (**A-1, C-1, E-1, G-1**) and leaves (**A-2, C-2, E-2, G-2**) were analyzed separately, in order to check their difference in composition. The chromatograms of the oils from the flowers and leaves were quite similar. However, some quantitative differences of components were observed (Figures 1-8).

The oils from the cultivated type constituted of *ca* 50 components. The compounds with percent composition greater than one are not more than seven, comprising 87 - 93% of the oils. The composition of the oils were observed to be simple mixtures, dominated by one compound which was identified to be l-linalool (**1**) (Tables 4 & 6). This monoterpene alcohol comprised 68.06 - 76.29% and 73.19 - 82.75% of the oil from the flowers and leaves, respectively. The sweet odour discharged when the leaves are crushed is attributable to linalool. The second major component was found to be a sesquiterpene hydrocarbon, germacrene D (5.01 - 10.86% in flower, and 6.74 - 9.48% in leaves). Ocimene, ß- myrcene, α- and ß-caryophyllene, α-farnesene and ß-cadinene were identified by GC-MS (Table 5).

Table 4: Compounds identified from the essential oils of cultivated *L. adoensis*

RT on SE-54	Compound	Methods of identification
3.16	α-Pinene	RT, PE
3.33	Camphene	RT, PE
3.56	ß-Pinene	RT, PE
3.66	ß-Myrcene	RT, PE, GC-MS
5.31	l-Linalool	RT, PE, NMR
7.10	Citral b	RT
9.05	α-Copaene	NMR, GC-MS
10.56	Germacrene D	NMR

Table 5: Compounds identified from the essential oils of cultivated *L. adoensis* by GC-MS

RT on SE-54	RT on Carbowax 50	Compound
3.66	11.15	ß-Myrcene
4.31	14.27	Ocimene
9.05	22.36	α-Copaene
9.68	25.16	ß-Caryophyllene
10.22	26.72	α-Caryophyllene
10.69	28.27	α-Farnesene
10.98	28.40	ß-Cadinene

Table 6: Chemical composition of the essential oils of cultivated *L. adoensis*

Compound	Percentage Composition							
	A-1	A-2	C-1	C-2	E-1	E-2	G-1	G-2
α-Pinene	0.01	----	0.01	0.01	0.03	0.06	0.02	----
Camphene	0.07	0.03	0.08	0.02	0.07	0.07	0.09	----
ß-Pinene	0.18	----	0.92	0.46	0.14	0.25	1.41	----
ß-Myrcene	0.14	0.38	0.47	0.46	0.25	0.25	0.44	0.15
Ocimene	0.29	0.70	0.92	1.34	1.07	0.24	2.27	0.06
l-Linalool	68.06	78.98	75.79	82.75	69.57	73.19	76.29	75.90
Ipsdienone	0.81	0.56	0.06	0.12	0.65	0.49	0.35	0.14
Citral b	1.54	1.49	0.04	0.32	2.32	2.56	0.67	0.84
α-Copaene	0.93	0.59	0.81	0.54	0.56	0.68	0.48	0.77
Germacrene D	10.86	7.50	9.43	6.74	8.24	9.48	5.01	9.17
ß-Caryophyllene	3.57	2.33	0.21	0.17	2.74	3.12	1.17	1.19
α-Caryophyllene	0.55	0.30	0.39	0.22	0.51	0.37	0.17	0.28
α-Farnesene	1.25	0.47	1.74	1.17	1.06	0.63	1.72	2.37
ß-Cadinene	1.07	0.46	0.97	0.52	0.89	0.58	0.43	0.59

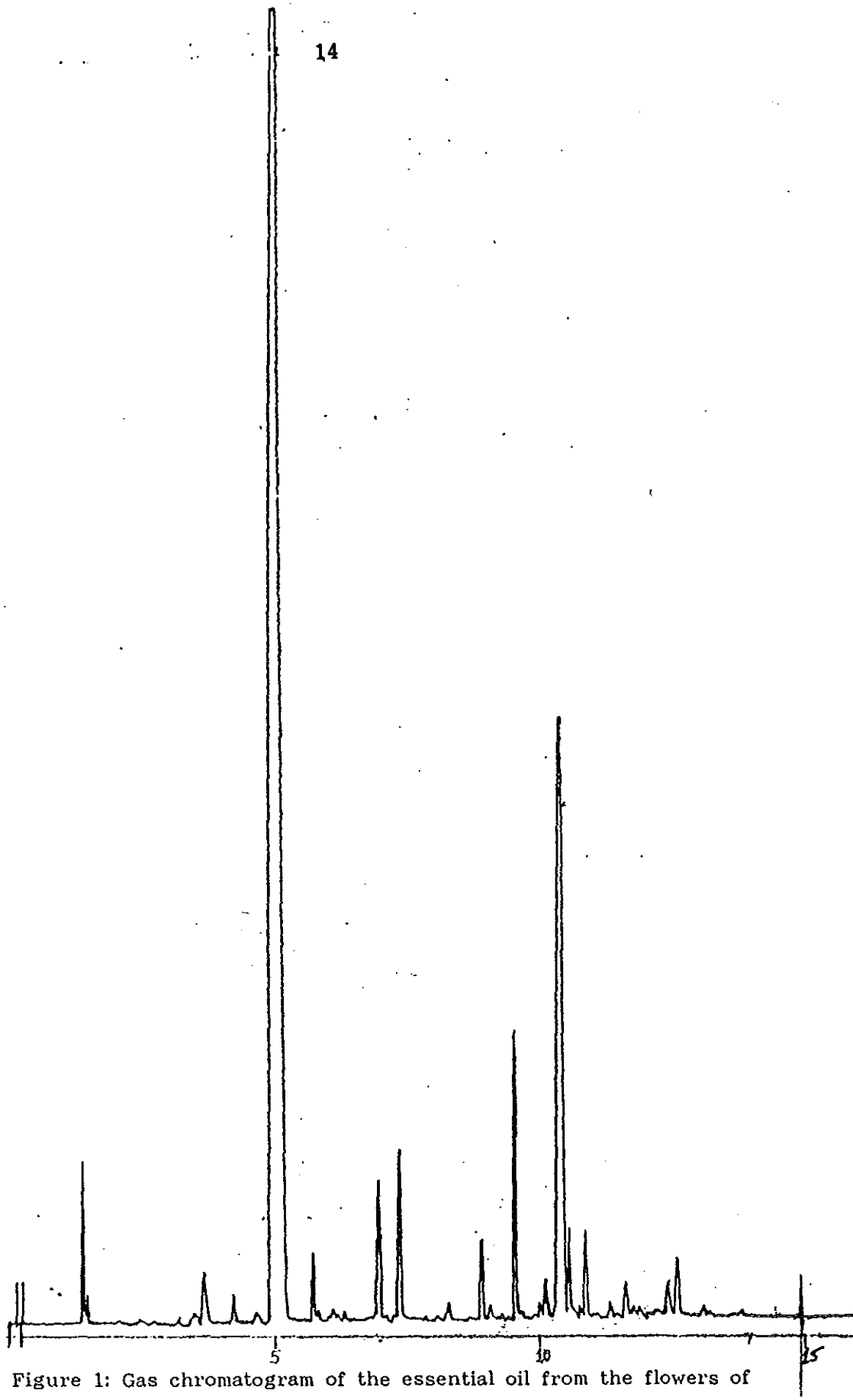


Figure 1: Gas chromatogram of the essential oil from the flowers of cultivated *L. adoensis* (A - 1)

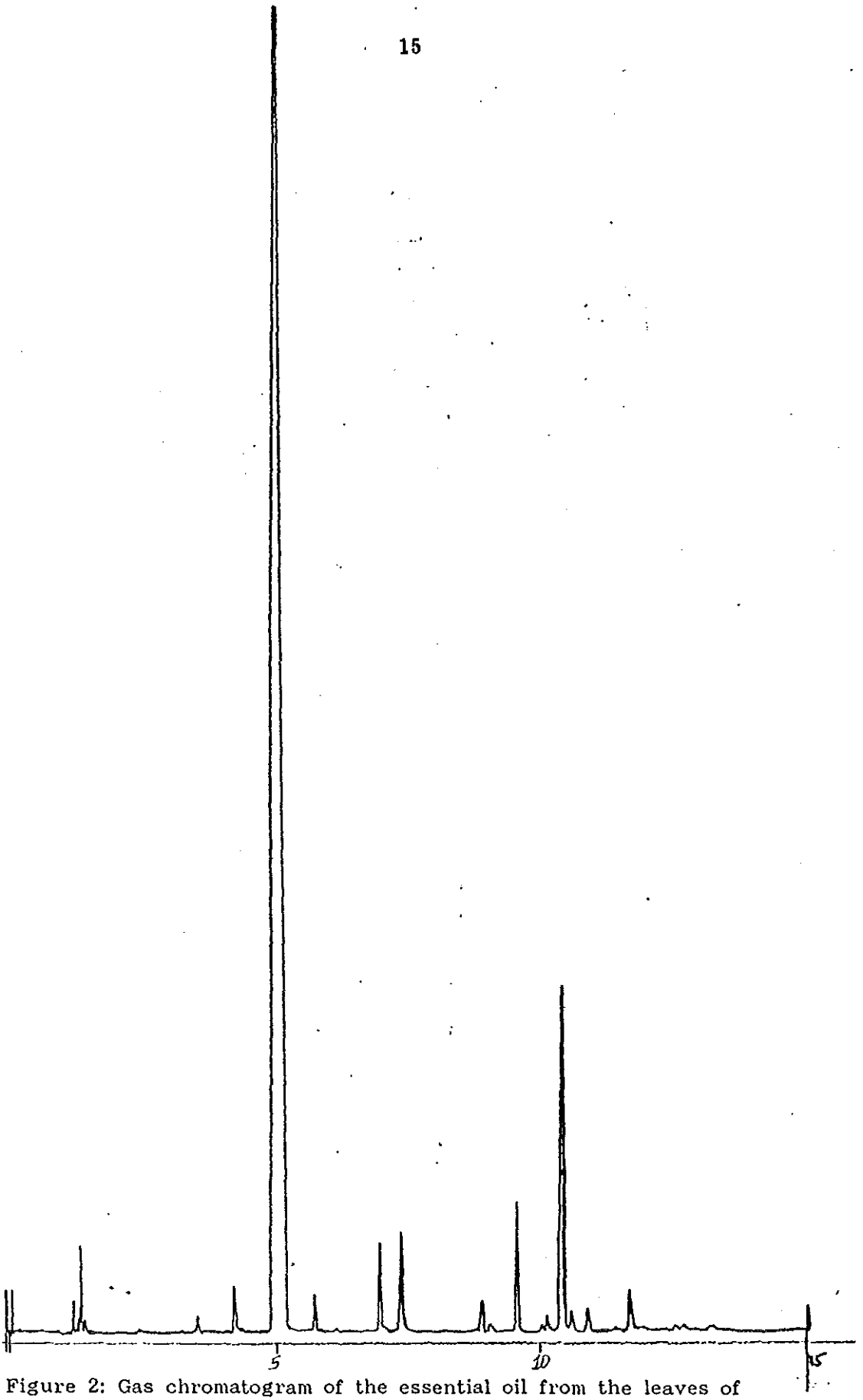


Figure 2: Gas chromatogram of the essential oil from the leaves of cultivated *L. adoensis* (A - 2)

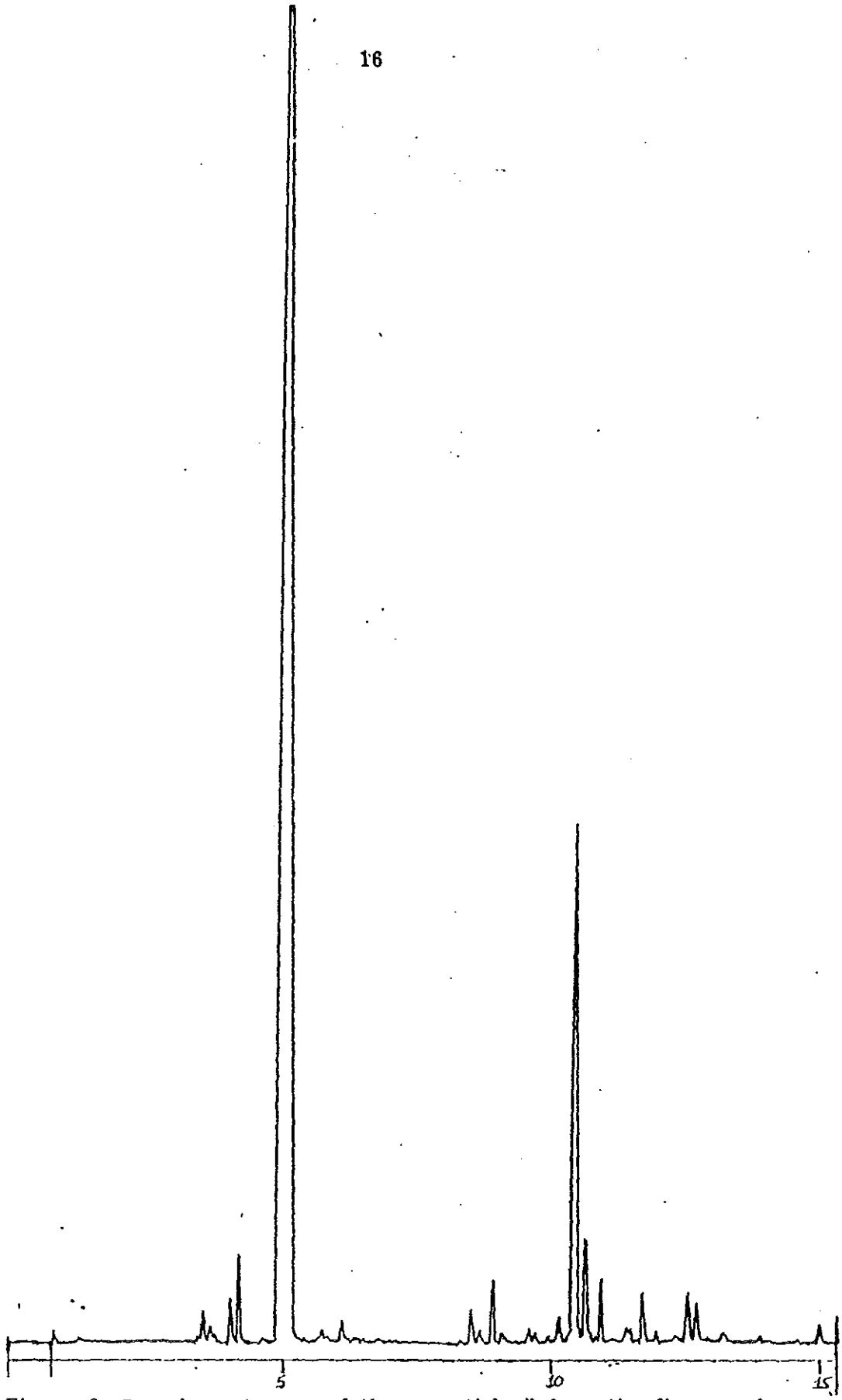


Figure 3: Gas chromatogram of the essential oil from the flowers of cultivated *L. adoensis* (C - 1)

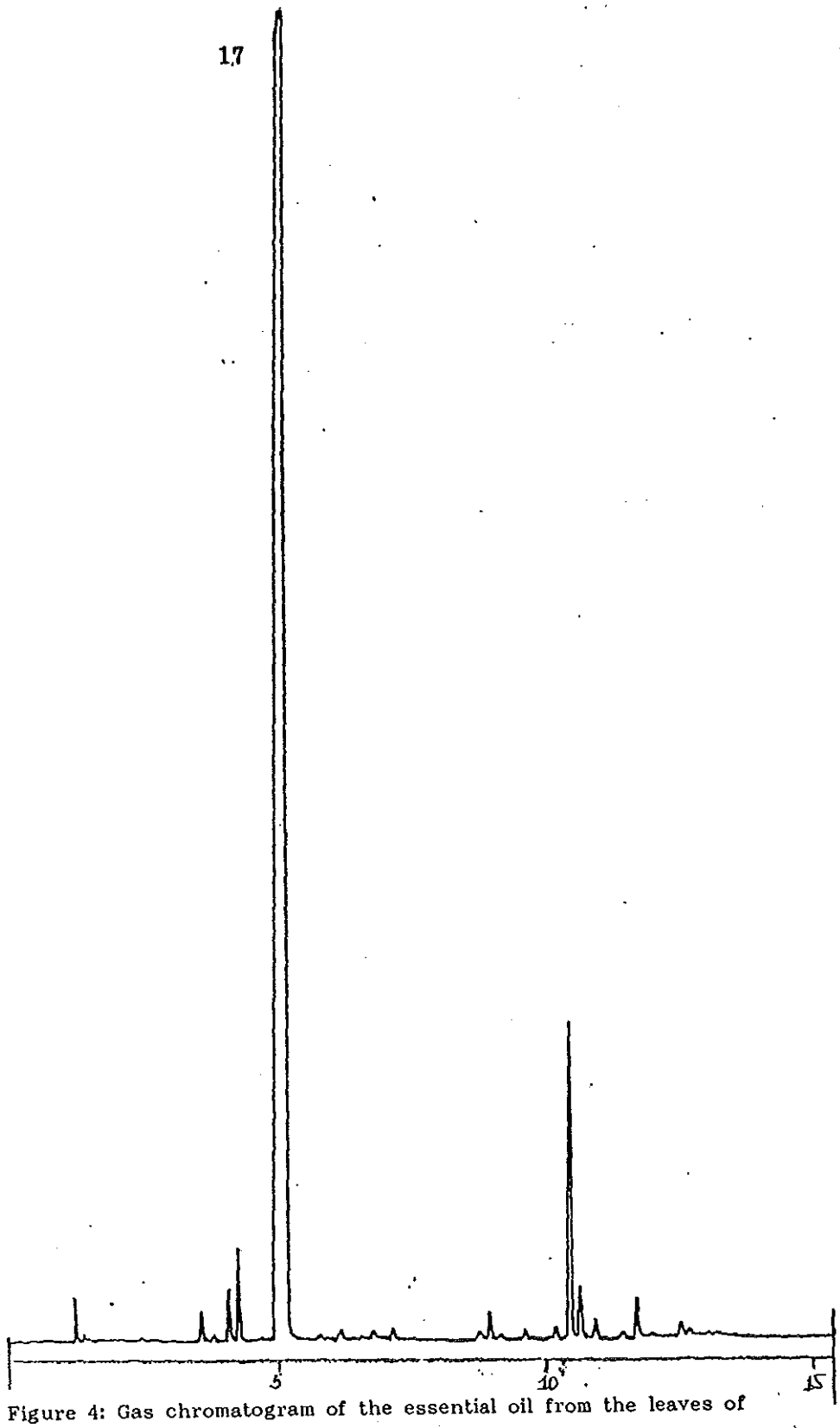


Figure 4: Gas chromatogram of the essential oil from the leaves of cultivated *L. adoensis* (C - 2)

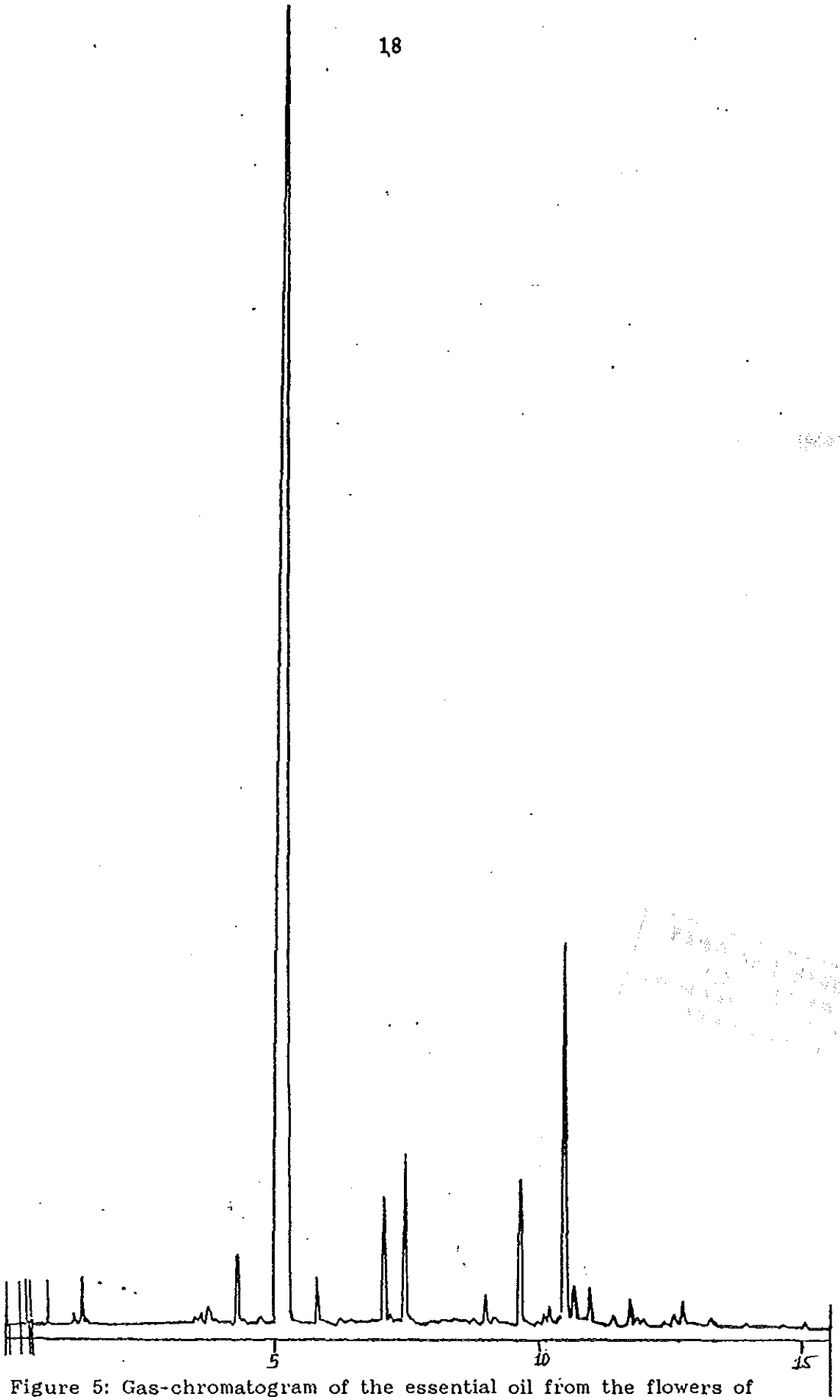


Figure 5: Gas-chromatogram of the essential oil from the flowers of cultivated *L. adoensis* (E - 1)

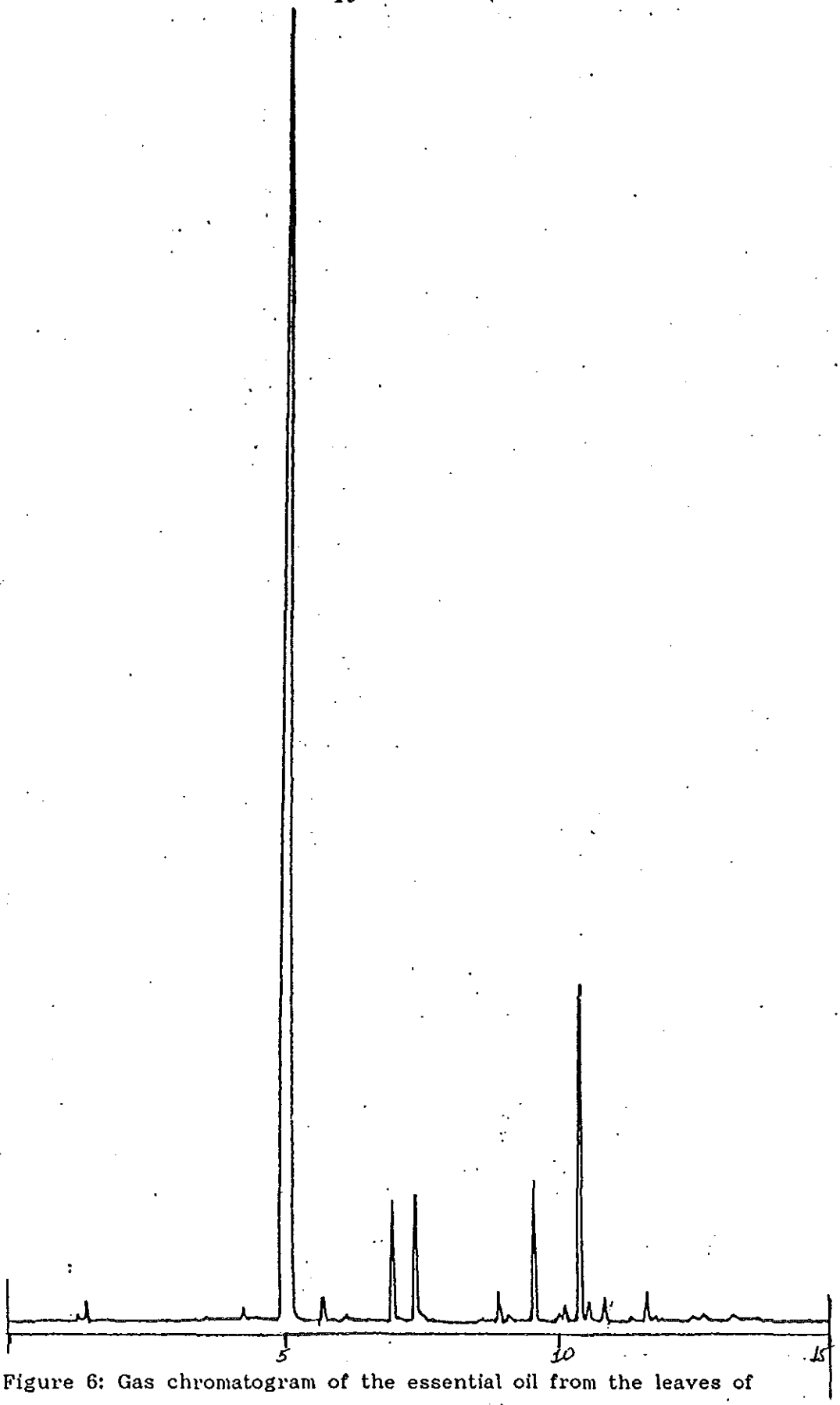


Figure 6: Gas chromatogram of the essential oil from the leaves of cultivated *L. adoensis* (E – 2)

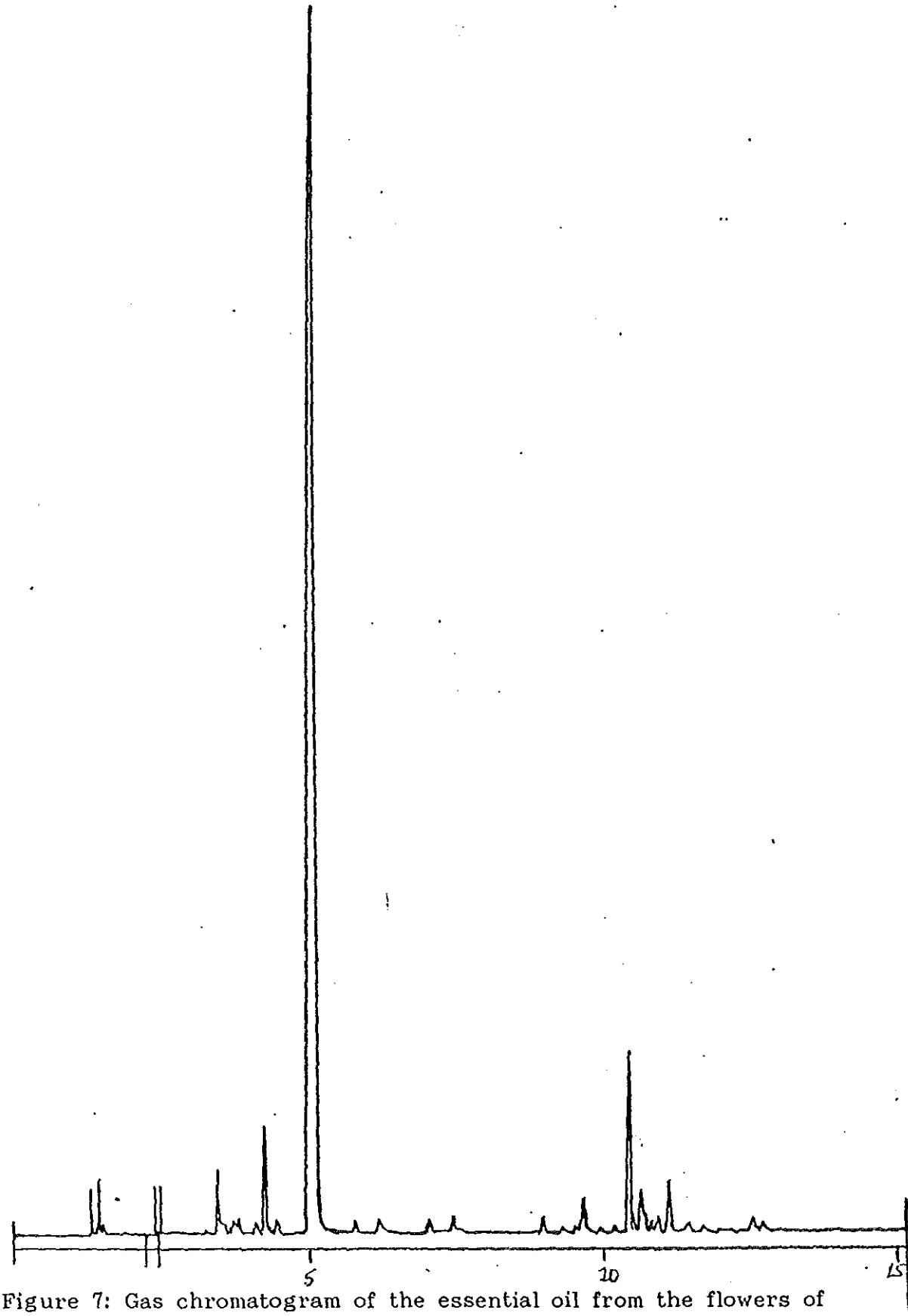


Figure 7: Gas chromatogram of the essential oil from the flowers of cultivated *L. adoensis* (G - 1)

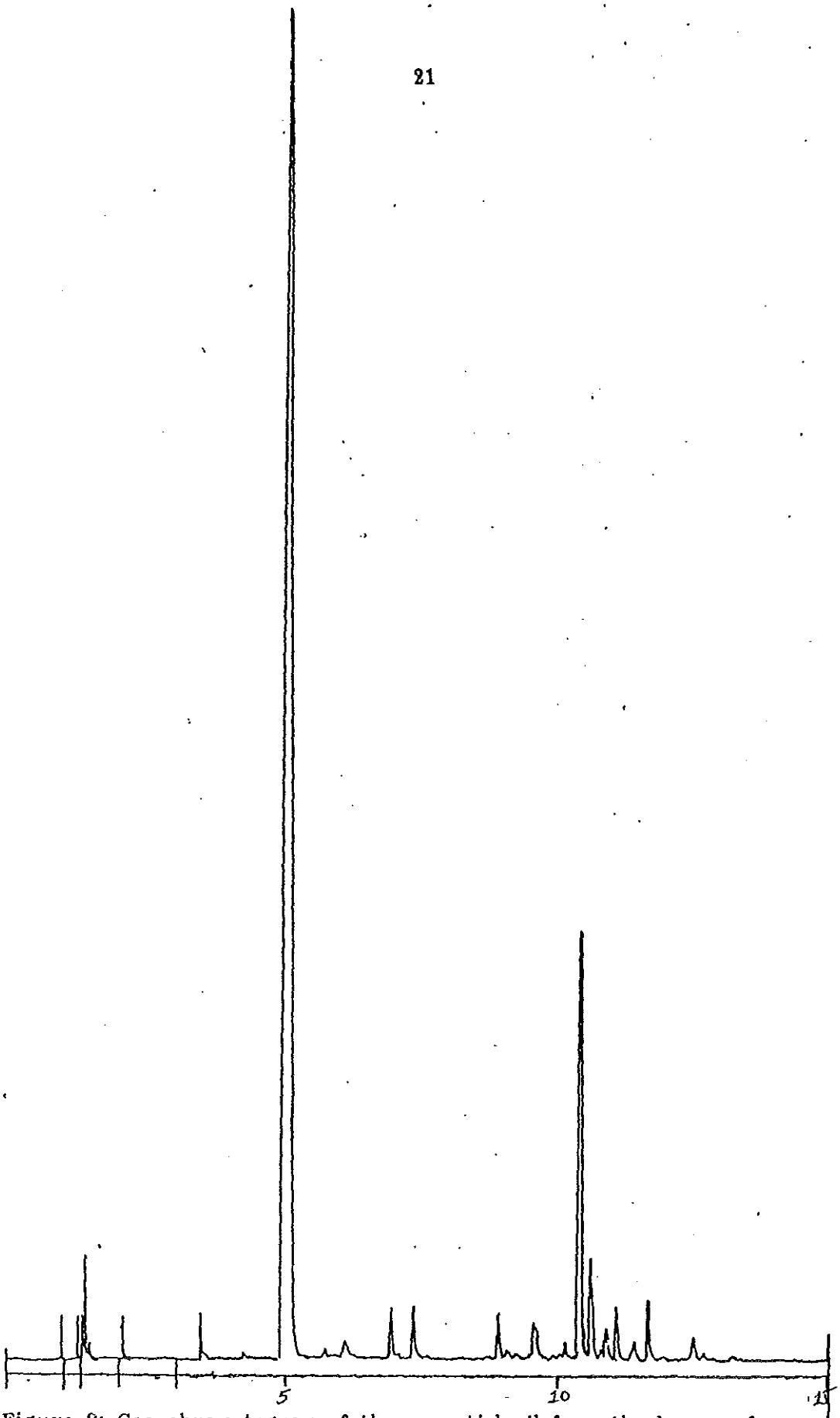


Figure 8: Gas chromatogram of the essential oil from the leaves of cultivated *L. adoensis* (G – 2)

L. Palfray *et al* [23] reported camphor content of 29.2% from the essential oils of *L. adoensis* grown in Senegal. Three other reports by Rabate and Laffitte [20-22] described the essential oils of *L. adoensis* from French West Africa as being composed of 33 to 43% camphor. Rovesti [19] had earlier reported 72% carvone from essential oil of *L. adoensis* from Ethiopia. Elakovich and Oguntimein [25] have analyzed *L. adoensis* from Nigeria. They observed no camphor or carvone in the oil, but found linalool to be the dominant component of the oils of both leaves and flowers, while the flowers contained more linalool than the leaves. Our findings for the cultivated *L. adoensis* are in agreement with those of Elakovich [25]. However, we found no thymol in the oils, and the oils from the leaves contained more linalool than those of the flowers.

2.2.2 Essential oils from the wild *L. adoensis*

The relatively complex chromatograms of the essential oils obtained from the flowers (**B-1, D-1, F-1, H-1**) and leaves (**B-2, D-2, F-2, H-2**) of the wild *L. adoensis* were found to be similar except for some differences in percentage composition of components (Figures 9-16). Each oil consists of *ca* 60 components and the compounds with percentage composition greater than one are about thirteen and they comprise 84 to 89% of the oils. Fairly large amounts of limonene, perillaldehyde, piperitenone, citral a, citral b, and ipsdienone characterize the oils (Tables 7 and 9). The fragrance of the leaves on bruising is reminiscent of limonene. This lemon like odour can be used to differentiate the wild from the cultivated which has a rather sweet fragrance. Analyses of the oil by GC-MS indicated the presence of α-copaene, ß-myrcene, α- and ß-caryophyllene, α- and ß-cadinene (Table 8).

The composition of the essential oils from the wild was found to be quite different from those of the cultivated *L. adoensis*. The absence of l-linalool in the wild while it is the major component in the cultivated type (Table 5) together with the absence of d-limonene, perillaldehyde, piperitenone, and citral a in the cultivated clearly differentiate the two types. Both contained appreciable amounts of the sesquiterpene hydrocarbons, germacrene D, α-

copaene, ß-cadinene, α- and ß-caryophyllene. The chemical compositions of the wild are als different from those previously reported [18-25] for this species.

Table 7: Compounds identified from the essential oil of wild *L. adoensis*

RT on SE-54	Compound	Methods of identification
3.16	α-Pinene	RT, PE
3.33	Camphene	RT, PE
3.56	ß-Pinene	RT, PE
3.66	ß-Myrcene	RT, PE, GC-MS
4.25	d-Limonene	RT, PE, NMR
5.83	Ipsdienone	NMR, MS
7.10	Citral b	NMR
7.58	Citral a	NMR
7.86	Perillaldehyde	NMR
8.73	Piperitenone	NMR
9.05	α-Copaene	NMR, GC-MS
10.56	Germacrene D	NMR

Table 8: Compounds identified from the wild *L. adoensis* by GC-MS

RT on SE-54	RT on Carbowax 50	Compound
3.66	11.15	ß-Myrcene
4.25	12.39	d-Limonene
9.05	22.15	α-Copaene
9.68	24.89	ß-Caryophyllene
10.22	26.67	α-Caryophyllene
10.73	27.63	α-Cadinene
10.98	28.38	ß-Cadinene

Figure 9: Gas chromatogram of the essential oil from the flowers of wild *L. adoensis* (B - 1)

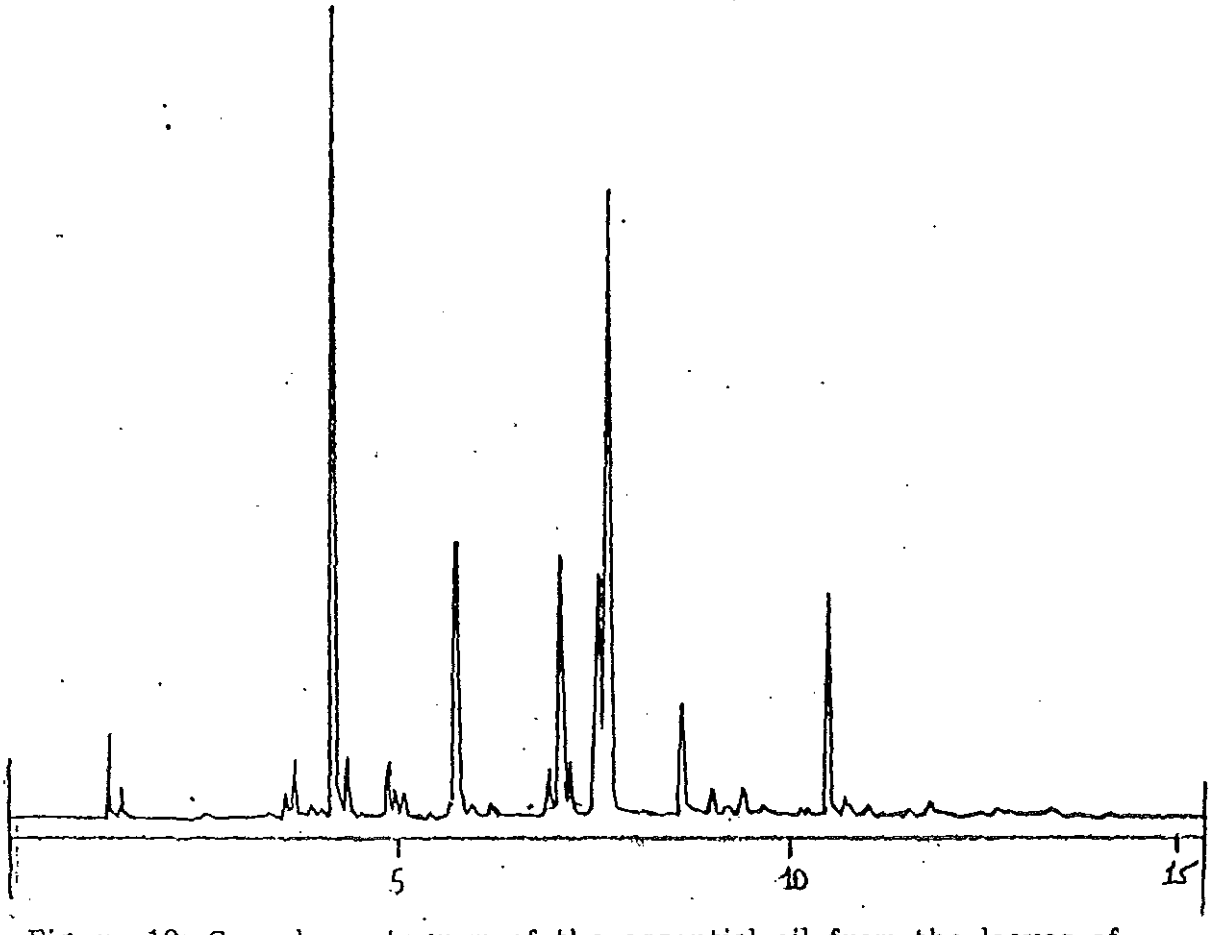


Figure 10: Gas chromatogram of the essential oil from the leaves of wild *L. adoensis* (B - 2)

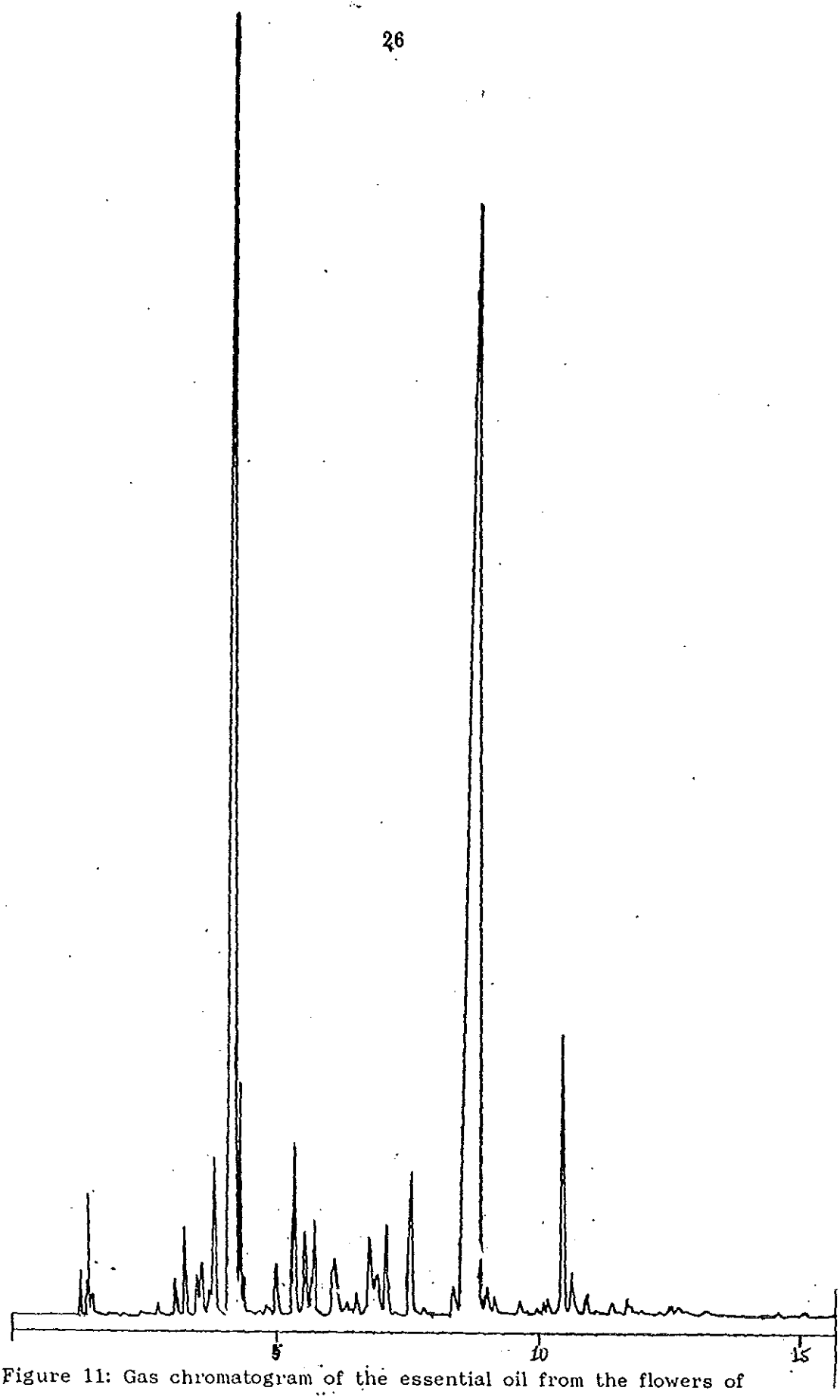


Figure 11: Gas chromatogram of the essential oil from the flowers of wild *L. adoensis* (D - 1)

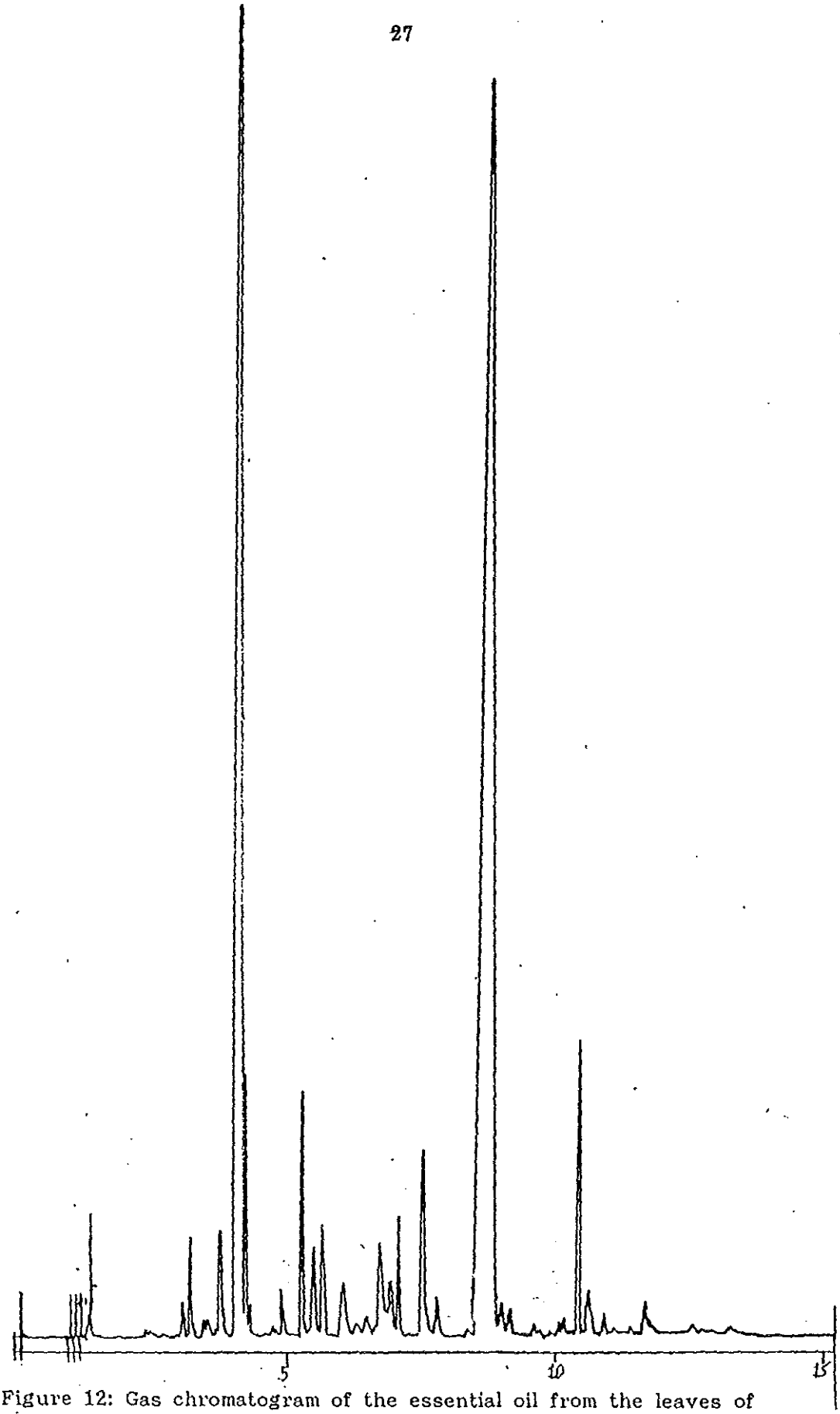


Figure 12: Gas chromatogram of the essential oil from the leaves of wild *L. adoensis* (D - 2)

Figure 13: Gas chromatogram of the essential oil from the flowers of wild *L. adoensis* (F - 1)

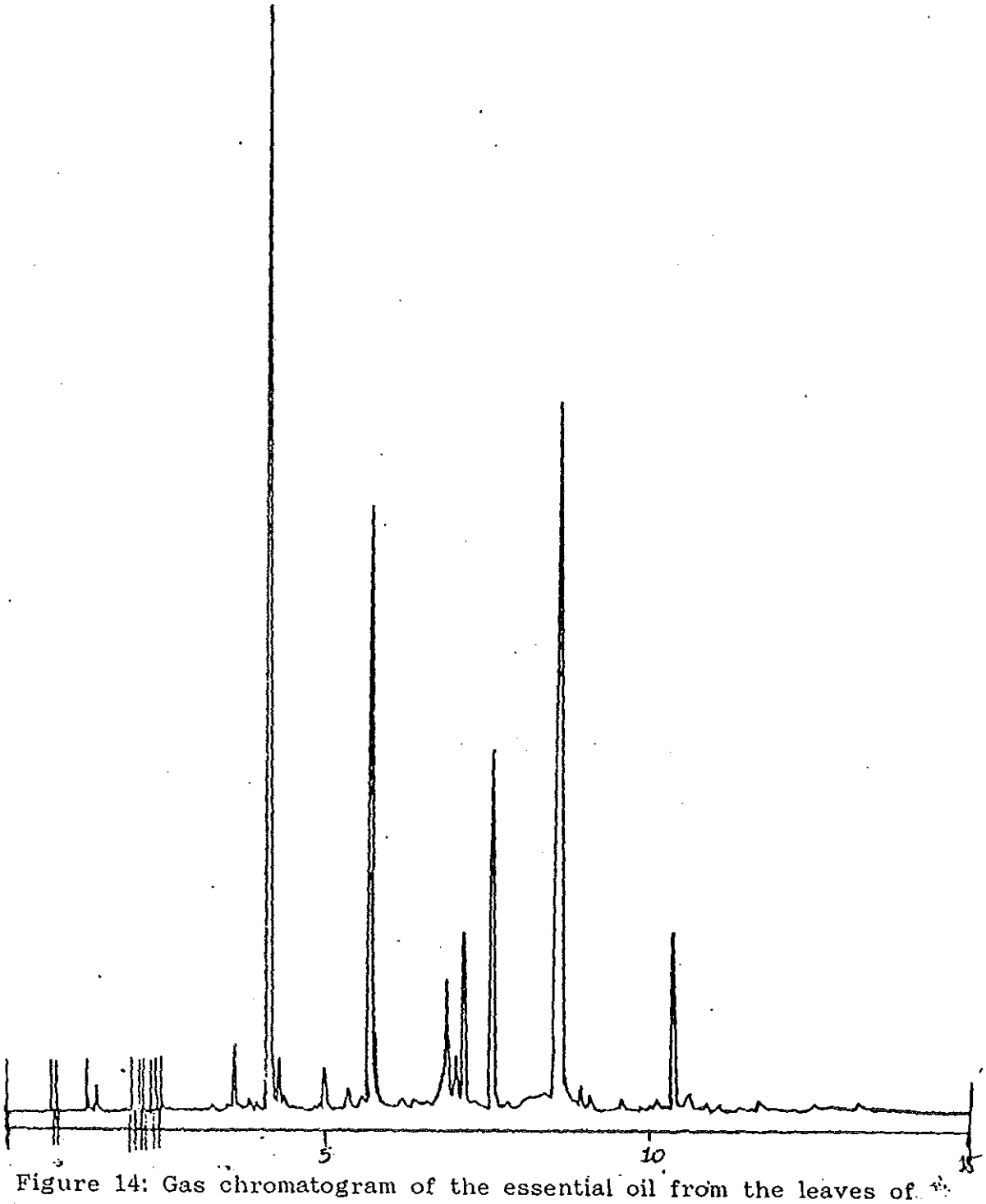


Figure 14: Gas chromatogram of the essential oil from the leaves of wild *L. adoensis* (F - 2)

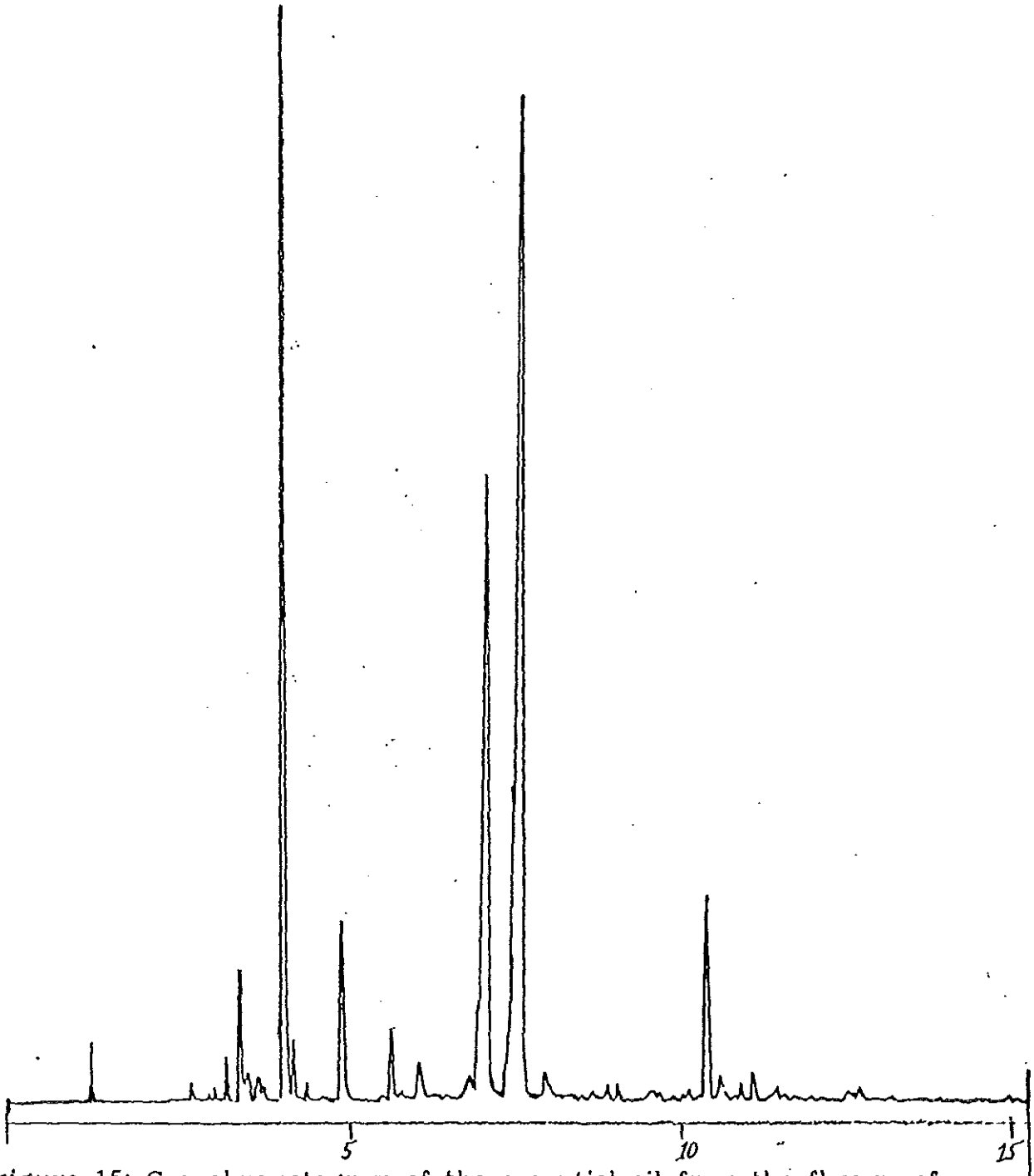


Figure 15: Gas chromatogram of the essential oil from the flowers of wild *L. adoensis* (H - 1)

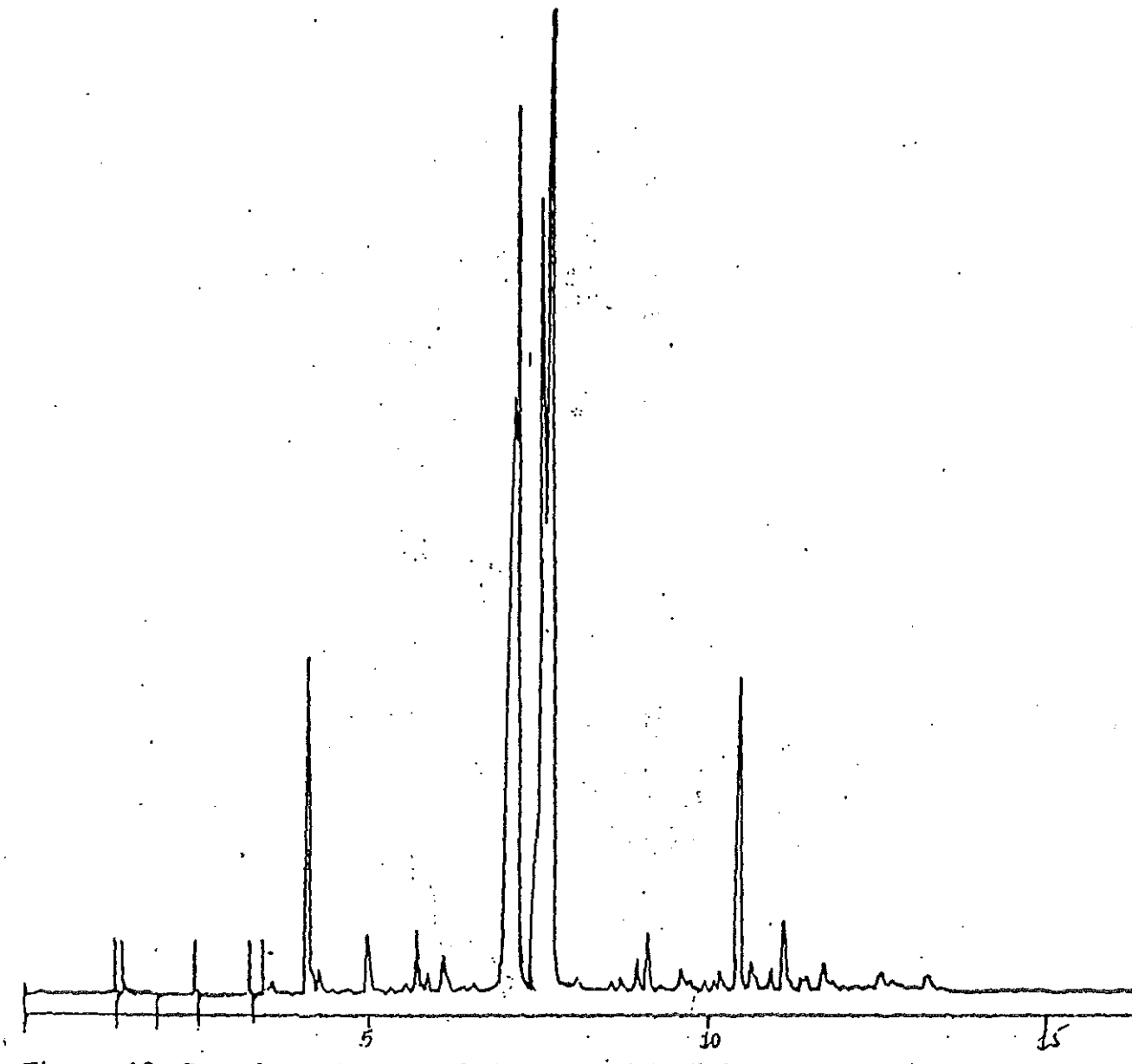


Figure 16: Gas chromatogram of the essential oil from the leaves of wild *L. adoensis* (H - 2)

Table 9: Chemical composition of the essential oils of Wild *L. adoensis*

Compound	Percentage Composition							
	B-1	B-2	D-1	D-2	F-1	F-2	H-1	H-2
α-Pinene	0.10	0.04	0.30	0.22	0.18	0.03	0.21	----
Camphene	0.28	0.12	0.67	0.61	0.45	0.10	0.54	----
ß-Pinene	0.67	0.57	0.37	0.13	0.38	0.14	1.67	0.05
ß-Myrcene	1.58	1.48	0.72	0.28	2.02	1.08	0.74	0.17
d-Limonene	15.74	16.63	32.73	32.30	27.82	22.22	17.67	3.44
Ipsdienone	10.42	7.21	1.27	0.92	12.04	14.89	1.24	0.81
Citral b	7.77	7.68	0.88	1.05	1.04	1.48	8.20	16.26
Citral a	15.12	9.72	1.92	2.08	5.80	8.41	9.30	18.85
Perillaldehyde	16.03	26.73	0.15	0.37	0.04	0.11	26.90	22.13
Piperitenone	5.64	4.17	41.21	44.48	25.69	27.86	0.21	0.15
α-Copaene	0.81	1.03	0.30	0.16	0.65	0.58	0.39	0.36
Germacrene D	4.68	5.43	2.95	2.83	3.65	3.37	3.43	4.24
ß-Caryophyllene	0.62	0.77	0.15	0.14	0.36	0.33	0.55	0.49
α-Caryophyllene	0.29	0.28	0.14	0.16	0.22	0.37	0.31	0.40
α-Cadinene	1.21	0.88	0.66	0.58	1.12	0.90	0.78	0.74
ß-Cadinene	0.50	0.35	0.27	0.18	0.33	0.37	0.42	0.38

2.3 Isolation and characterization of constituents

The essential oils were obtained from the plant materials by hydrodistillation using a Clevenger apparatus. Isolation of compounds from the essential oils of the cultivated (A-2) and wild (B-2)

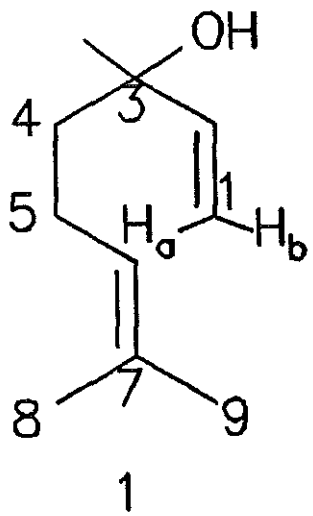
L. adoensis were conducted using column chromatography, chromatotron, and preparative TLC, as described in the Experimental section.

2.3.1 l-Linalool (1)

This light yellow monoterpene alcohol was obtained from the ethyl acetate fraction of the cultivated *L. adoensis*. Optical rotation measurement indicated the compound to be laevorotatory. The IR spectrum displayed absorption bands for a hydroxyl (3450 cm^{-1}), and a vinyl group (3080, 3050, 980 and 900 cm^{-1}). The presence of vinyl protons (ABX pattern, J_{AB} = 1.8 Hz, J_{AX} = 16 Hz, J_{BX} = 10.8 Hz), two methyl groups on a double bond (δ 1.60, 1.67), and another methyl group (δ 1.26) attached to an sp^3 hybridized carbon atom was evident from the ^1H NMR spectrum. The ^{13}C NMR spectrum (Table 10) displayed 10 carbon resonances corresponding to three primary, two secondary, two tertiary and two quaternary carbon atoms.

Table 10: ^{13}C NMR data of linalool (1) (22.5 MHz, $CDCl_3$)

C	δ_{ppm}
1	112.9
2	146.8
3	74.7
3-CH_3	29.2
4	43.8
5	24.3
6	125.9
7	133.1
8	18.9
9	26.9

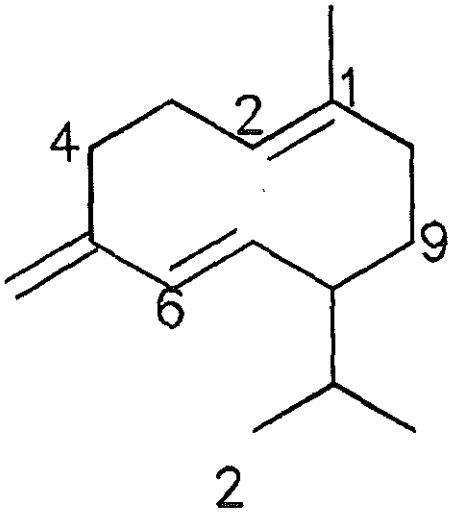


The above spectroscopic findings were found to be consistent with structure **1**. The identity of compound **1** was further established by comparison with an authentic sample using GC. The spectroscopic data obtained agree very well with that reported for l-linalool in the literature [27, 28].

Linalool is one of the most industrially important monoterpene alcohols used extensively in perfumes, for soaps and cosmetics and in flavours. Its odour resembles that of fresh flowers, like lavender and bergamot. Moreover, linalool and its precursors are indispensable for the manufacture of vitamins A and E [29].

2.3.2 Germacrene D (2)

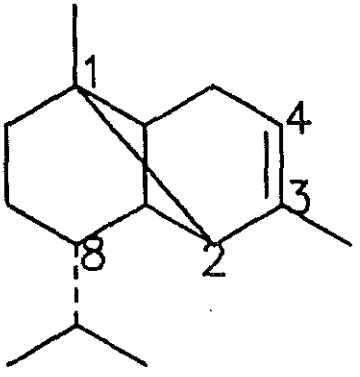
Compound **2** was isolated from the hexane fraction of cultivated *L. adoensis*. It had a retention time of 10.56 and was presumed to be a sesquiterpene from the RT. The MS displayed an intense molecular ion peak at *m/z* 204. The base peak appeared at *m/z* 161 as a result of loss of the isopropyl group. Other prominent peaks were at *m/z* 119, 105, 91, 81, 77 and 41.
The IR spectrum showed absorption bands for an isopropyl group (1380 cm^{-1}), terminal methylene group (1640, 880 cm^{-1}) and a *trans*-disubstituted double bond (970 cm^{-1}).



The ^{1}H NMR spectrum revealed the presence of an isopropyl group (δ 0.89, *d*, 6H), a terminal methylene group (δ 4.77), a methyl group on a double bond (δ 1.52), a disubstituted double bond (AB part of ABX type signals at δ 5.24 and 5.82) and a trisubstituted double bond (δ 5.0-5.5). Structure **2** is consistent with the above spectroscopic findings. Comparison of the spectroscopic data with those reported in the literature [30, 31] for germacrene D revealed a very close agreement.

2.3.3 α-Copaene (3)

This sesquiterpene hydrocarbon was isolated from the hexane fraction of the cultivated *L. adoensis*. The MS exhibited an intense molecular ion at *m/z* 204 and a base peak at 105. Other prominent peaks were observed at *m/z* 119, 161, 93 and 41. The ^{1}H NMR showed the presence of an isopropyl group (δ 0.86, *d*), quaternary methyl (δ 0.78), a methyl on a double bond and a proton on a trisubstituted double bond. The GC-MS spectrum revealed a peak matching of this compound to α-copaene. The ^{1}H NMR data acquired was similar with that reported for α-copaene in the literature [32].



3

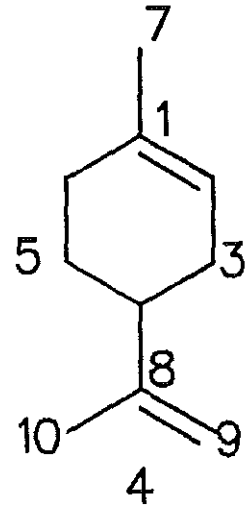
2.3.4 d-Limonene (4)

This hydrocarbon monoterpene was isolated as a colourless oily liquid from the hexane fraction

of the wild *L. adoensis*. The compound was found to be dextrorotatary and the value obtained was comparable with the reported [33] value for d-limonene. The identity of the compound was first established by GC based on its RT and peak enhancement method using reference limonene. The IR spectrum indicated a band at 880 cm^{-1} attributable to a terminal methylene group. The presence of methyl groups on double bond (δ 1.67 and 1.75), terminal methylene protons (δ 4.69), and a proton on a trisubstituted double bond (δ 5.39) was evident from the ^{1}H NMR spectrum. The ^{13}C NMR spectrum (Table 11) displayed 10 carbon resonances. The spectroscopic data presented above was consistent with structure **4**, and is in close agreement with those described in the literature [34, 35] for d-limonene.

Table 11: ^{13}C NMR data of limonene (**4**) (22.5 MHz, $CDCl_3$)

C	δ_{ppm}
1	133.7
2	120.9
3	30.9
4	41.4
5	28.3
6	31.1
7	23.3
8	150.2
9	108.5
10	20.8



Limonene is an important and widespread terpene. It is extensively used in the perfumery and flavour industries and in the manufacture of polymers and adhesives [33]. The (+)-form is the major constituent of oil of bergamot, caraway and lemon. The (-)-form is a constituent of pine needle oil.

2.3.5 Ipsdienone (5)

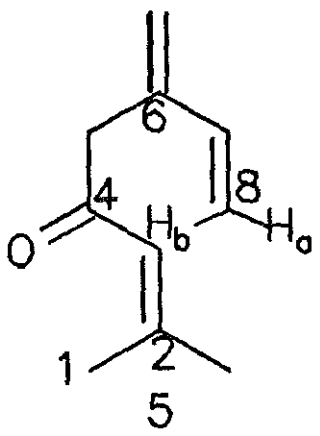
This compound was isolated from the ethyl acetate fraction of the oil of the wild *L. adoensis*, and it was found to be optically inactive. Small quantity of the compound has also been isolated from the cultivated type. The MS displayed a very weak molecular ion at *m/z* 150. The base peak appeared at *m/z* 83 as a result of α cleavage. The UV spectrum revealed the presence of a conjugated ketone (λ_{max} at 225 and 286 nm). The presence of vinyl protons (δ 5.09 and 5.15, ABX pattern, J_{AB} = 1.6 Hz, J_{AX} = 14.6 Hz, J_{BX} = 8.3 Hz), a proton on a trisubstituted double bond (δ 6.15), and terminal methylene protons (δ 5.25) was derived from the ^{1}H NMR spectrum. The ^{1}H NMR data has a close similarity with that of ipsenone (**6**), isolated from *L. multiflora* [36] (Table 12). However, the resonance observed for the methyl groups of compound **5** are relatively down field suggesting the presence of a 2,3-double bond. This was further supported by the presence of an olefinic proton signal at δ 6.15 which is assignable to H-3. The ^{13}C NMR spectrum (Table 13) revealed 10 carbon resonances. The signal at δ 197.9 is assignable to C-4 and further substantiates the presence of an α,ß-unsaturated carbonyl group. Only three carbon resonances appear in the aliphatic region and are consistent with one methylene and two methyl groups. The remaining six carbon resonances appear in the region between δ 114 and 156 and establish the occurrence of three double bonds in the molecule. The above spectroscopic data allowed the assignment of structure **5** to this compound. Compound **5** (ipsdienone) has earlier been synthesised [37, 38]. The reported ^{1}H NMR data for the synthetic compound [39] agree very well with the data for the natural product isolated (Table 11). 2-methyl-6-methylene-2,7-octadiene-4-one (ipsdienone) is important in the synthesis of ipsdienol, a component of the sex attractant produced by *Ips confusus* [37]. To the best of our knowledge, this is the first finding of the ketone ipsdienone from a natural source.

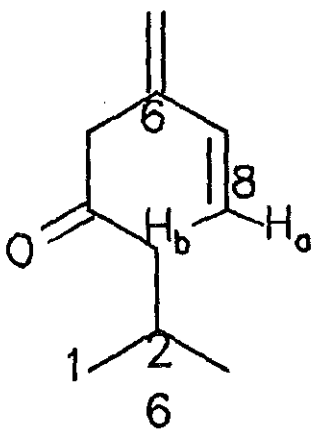
Table 12: ^{1}H NMR data of **5** (90 MHz, $CDCl_3$) and ipsenone (6) (200MHz, $CDCl_3$)

Proton	**5**	Ipsenone	Ipsdienone
H-1	2.14	0.89	2.12
2-C$\underline{H}_3$	1.88	0.89	1.87
H-2	—	2.13	—
H-3	6.15 (1H)	2.33 (2H)	6.16
H-5	3.28	3.27	3.28
6-C$\underline{H}_2$	5.25	5.25	5.32-4.95
H-7	6.41	6.42	6.44
H-8_a	5.09	5.10	5.32-4.95
H-8_b	5.15	5.13	5.32-4.95

Table 13: ^{13}C NMR data of Compound **5** (22.5 MHz, $CDCl_3$)

C	δ_{ppm}
1	27.5
2-$\underline{C}H_3$	20.7
2	138.5
3	122.9
4	197.9
5	48.1
6-CH_2	114.8
6	155.6
7	141.1
8	119.3





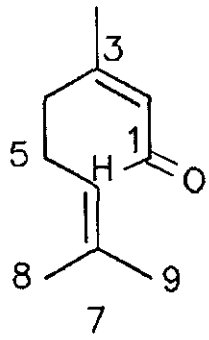
2.3.6 Citral b (7)

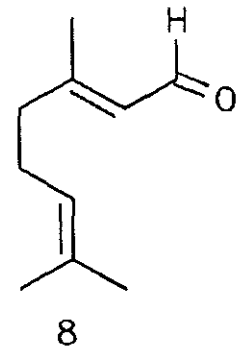
This compound was isolated from the EtOAc fraction of the wild *L. adoensis* and is also present in the cultivated type in a smaller amount. The presence of an aldehyde proton (δ 9.89), three methyl groups on double bond (δ 1.60, 1.69, 1.98), two protons on trisubstituted double bonds (δ 5.11, 5.87) was deduced from the ^{1}H NMR spectrum. The ^{1}H NMR data is consistent with structure (7) and agrees very well with that reported in the literature [40] for citral b.

2.3.7 Citral a (8)

This compound was isolated from the EtOAc fraction of the wild *L. adoensis*. The ^{1}H NMR spectrum indicated the presence of an aldehyde proton (δ 9.98), three methyl groups on double bonds (δ 1.60, 1.69, 2.18) and two olefinic protons on tri-substituted double bonds (δ 5.03, 5.87). The ^{1}H NMR data is in very close agreement with that reported for citral a in the literature [40].

Citral has a pleasant lemon odour, and finds extensive application as a flavouring and perfumery ingredient. It is also an important starting material for the synthesis of ionones and vitamin A [41].



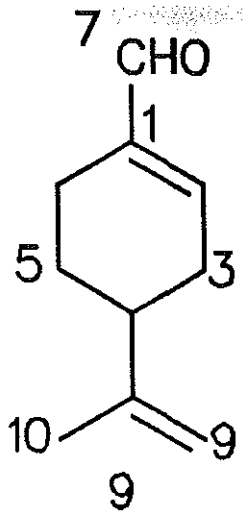


2.3.8 Perillaldehyde (9)

This compound was obtained from the EtOAc fraction of the wild *L. adoensis*. The UV spectrum indicated the presence of an α,ß-unsaturated carbonyl group. The presence of an aldehyde group (2730, 1690 cm^{-1}) and a disubstituted methylene group (890 cm^{-1}) was derived from the IR spectrum.

Table 14: ^{13}C NMR data of perillaldehyde (9) (22.5 MHz, $CDCl_3$)

C	δ_{ppm}
1	141.6
2	149.6
3	31.9
4	41.0
5	26.7
6	21.8
7	193.3
8	148.4
9	109.5
10	20.6



The ^1H NMR spectrum showed an aldehyde proton (δ 9.24), terminal methylene protons (δ 4.55), a proton on a trisubstituted double bond (δ 6.64) and a methyl group on double bond (δ 1.55). The presence of an α,ß-unsaturated carbonyl group, two double bonds and five aliphatic carbon atoms was further confirmed by the ^{13}C NMR spectrum (Table 14). Based on the above spectroscopic data, structure **9** was assigned to this compound. Comparison of the spectroscopic data with those reported in the literature [34, 40] for perillaldehyde revealed a very close agreement.

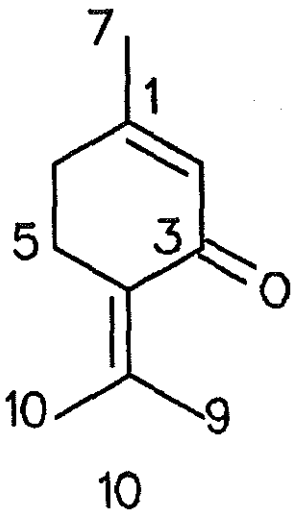
2.3.9 Piperitenone (10)

This compound was isolated from EtOAc fraction of the wild *L. adoensis*. The presence of ar

α,ß-unsaturated ketone was evident from the UV and IR spectra. The ^{1}H NMR spectrum revealed the presence of three methyl groups on double bonds (δ 1.81, 1.88, 2.04) and a proton on a tri-substituted double bond (δ 5.84).

Table 15: ^{13}C NMR data of piperitenone (**10**) (22.5 MHz, $CDCl_3$)

C	δ_{ppm}
1	158.5
2	129.3
3	191.1
4	128.9
5	28.0
6	31.9
7	23.5
8	142.3
9	22.4
10	22.8



The ^{13}C NMR spectrum (Table 15) indicated 10 carbon resonances corresponding to three primary, two secondary , one tertiary and four quaternary carbon atoms. The above spectroscopic data led to the assignment of structure **10** to this natural product. The ^{1}H and ^{13}C NMR data agree very well with those reported for piperitenone in the literature [34 & 40].

2.3.10 α-Copaene (3)

This sesquiterpene hydrocarbon was isolated from the hexane fraction of the wild *L. adoensis*. The RT, ^{1}H NMR and MS spectra obtained for the compound were similar with those of α-copaene isolated from the cultivated type. GC-MS peak matching also indicated the compound to be α-copaene.

2.4 Morphological differences of the leaves

The wild and cultivated *L. adoensis* plants are very similar. Morphological studies [1 conducted on the leaves of the wild and cultivated *L. adoensis* indicated that the wild type h densely reticulated leaves with trichomes of multicellular base, while the leaves of the cultivat are sparsely reticulated with trichomes of unicellular base (Figure 17). It was also noted that t leaves of the wild are coarse and those of the cultivated are relatively smooth. Thus one wou be able to differentiate the two types by the texture of the leaves.

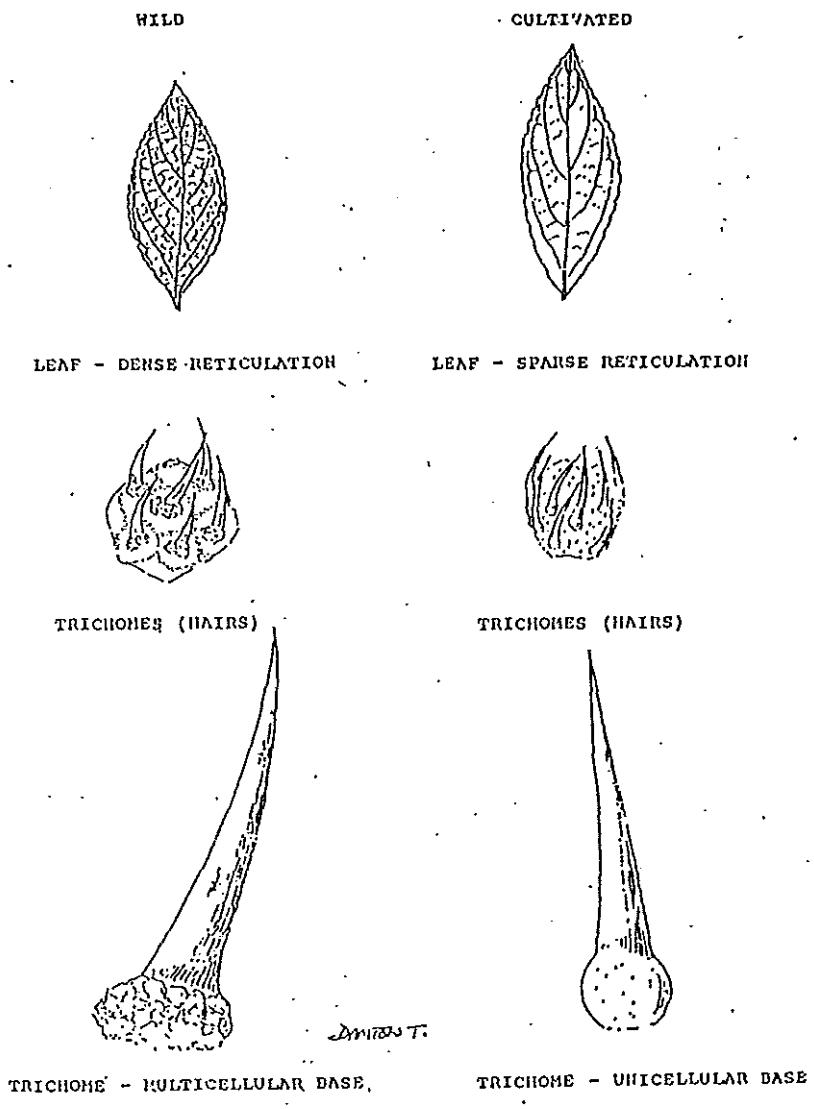


Figure 17: Morphological differences of the wild and cultivated L. adoensis

2.5 CONCLUSION

The chemical investigation on the essential oils of the wild and cultivated *L. adoensis* revealed the two types to be markedly different in their physical characteristics and chemical composition. Morphological study also indicated the leaves to be different. Some of the major differences between the two types are summarized below.

	Cultivated	Wild
Local name	*Kosseret, Koshonota, Shokonota, Kessenet*	*Kesse, Kussaye, Kessaye*
Odour	sweet (linalool)	lemony (limonene)
Optical rotation	laevorotatory	dextrorotatory
Leaves	soft	coarse
Reticulation of leaves	dense	sparse
Hairs	multicellular base	unicellular base

Fourteen components comprising 88-95% of the oil from the cultivated and 16 components constituting 68-86% of the oil from the wild were identified. Among these, ipsdienone is reported for the first time from a natural source.

The difference in the flavour of the wild and cultivated *L. adoensis* claimed by the ethnic groups can now be explained in terms of the difference in the chemical composition of their essential oils. The sweet fragrance of the cultivated type is attributable to linalool while the lemony odour of the wild is predominantly due to limonene.

It is now clear that the two types of *L. adoensis* are chemically and morphologically different. Therefore, there is a sound scientific basis for further taxonomic study on *L. adoensis*.

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: 70°C to 200°C at 10°C/min

FID detector: 260°C

Injector block: 210°C

GC-MS: VG Analytical, Model VG 12-250

MS: VG Analytical, Model 7070 E-HF

UV: Milton Roy, Model 1001

IR: Perkin Elmer 727B

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 (Merck), coated on glass plates

Column chromatography: Silica gel 60 (Merck); silica gel impregnated with silver nitrate

Plant material

The wild and cultivated *L. adoensis* samples were collected from four areas in Shoa Administrative Region.

Sample A : (Cultivated), collected from Addis Ababa, Gulelae area, Higher 25, Kebele 01 (Alt. 2500 m) on Sept. 28, 1991; Voucher no. Sebsebe D. and Nigist A. 2939.

Sample B: (Wild), collected from Shoa Adm. Region, 32 km from Addis Ababa towards Ambo, just outside Menagesha town (Alt. 2500 m) on Oct. 7, 1991; Voucher no. Sebsebe D. and Nigist A. 2942.

Sample C: (Cultivated), collected from Shoa Adm. Region, Butajira town market, 134 km from Addis Ababa towards Hossaina (Alt. 2100 m) on Oct. 12, 1991; Voucher no. Sebsebe D., Nigist A. and Ermias D. 2956.

Sample D: (Wild), collected from Shoa Adm. Region, 1 km from Bui towards Butajira, 105 km from Addis Ababa (Alt. 2070 m) on Oct. 12, 1991; Voucher no. Sebsebe D., Nigist A. and Ermias D. 2955.

Sample E: (Cultivated), collected from Shoa Adm. Region, Sodo Gurage, 10 km off the main Addis Ababa - Butajira road (Alt. 2200 m) on Oct. 13, 1991; Voucher no. Asnaketch in Sebsebe D. 2960.

Sample F: (Wild), collected from Shoa Adm. Region, 70 km from Addis Ababa to Butajira between Lemen and Tiya (Alt. 2150 m), on Oct. 12, 1991; Voucher no. Sebsebe D., Nigist A. and Ermias D. 2954.

Sample G: (Cultivated), collected from Shoa Adm. Region, 186 km west of Addis Ababa, 7 km east of Ghedo (Alt. 2550 m), on Oct. 12, 1991; Voucher no. Ensermu K. 2301.

Sample H: (Wild), collected from Shoa Adm. Region, 5 to 6 km west of Ghedo (Alt. 2250 m) on Oct. 12, 1991; Voucher no. Ensermu K. 2293.

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-1, B-1, C-1, D-1, E-1, F-1, G-1, H-1**) and (**A-2, B-2, C-2, D-2, E-2, F-2, G-2, H-2**) were obtained from the flowers and the leaves, respectively by hydrodistillation.

Isolation

Essential oil from cultivated *L. adoensis*: 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. The oxygenated fraction was further fractionated on a silica gel column with 10% ethyl acetate in petroleum ether. A total of 35 fractions each of *ca* 10 ml were collected. Fraction 9 afforded linalool (**1**) which was obtained in a pure form. The hydrocarbon fraction was applied on a chromatotron, and small fractions were collected using hexane as eluent. Fractions 15 -17 afforded germacrene D (**2**). The hydrocarbon fraction was further applied on a chromatotron with silver nitrate impregnated silica gel plate and eluted with 25% CCl_4 in hexane. Small fractions were collected and fractions 2 - 4 gave α-copaene (**3**).

Essential oil from wild *L. adoensis*: 2.4 gm of the oil was fractionated on a silica gel column into hydrocarbon and oxygenated fractions using hexane and ethyl acetate, respectively. The oxygenated fraction was chromatographed on a silica gel column and eluted with 10% ethyl acetate in petroleum ether. Approximately 10 ml fractions were collected. Fraction 2 and fractions 23-29, after purification by preparative TLC each gave single compounds (**5**) and (**10**), respectively. Fractions 4-7 from CC were applied on a chromatotron and eluted with 10% ethyl acetate in petroleum ether. Small fractions of *ca* 1 ml each were collected. Fractions 16-19 and fraction 24 comprised of single compounds (**9**) and (**7**), respectively. Fractions 8-14 from the CC were applied on a chromatotron and eluted with 10% ethyl acetate in petroleum ether. Approximately 1 ml fractions were collected. Fractions 21-23 afforded a single compound (**8**). The hexane fraction was applied on 15% silver nitrate impregnated silica gel column and eluted with 20% CCl_4 in petroleum ether. About 10 ml fractions were collected. Fractions 25-35, and 14-16, each contained single compound (**4**) and (**3**), respectively.

Linalool (1)

Light yellow oily liquid; RT 5.31 min; $[\alpha]^{20}_{D}$: - 32.8°; IR ν_{max} cm^{-1}: 3450, 3080, 3050, 2980, 2930, 2850, 1450, 1375, 1100, 980, 900; ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.26 (3H, *s*, H-3), 1.60 (3H, *s*, H-8), 1.67 (3H, *s*, H-9), 2.05 (1H, -OH), 1.10-2.28 (H-4, H-5), 5.00 (1H, *t*, H-6), 5.10 (1H, *dd*, J = 10.8, 1.8 Hz, H-1_b), 5.19 (1H, *dd*, J = 16, 1.8 Hz, H1-$_a$), 5.89 (1H, *dd*, J = 16, 10.8 Hz, H-2); ^{13}C NMR (22.5 MHz, $CDCl_3$): See Table 10.

Germacrene D (2)

Light yellow oily liquid; RT 10.56 min; $[\alpha]^{21}_{D}$: - 141.2°; IR ν_{max} cm^{-1}: 3020, 2965, 2940, 2880, 1665, 1640, 1450, 1380, 1360, 970, 880; ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.89 (6H, *d*, J = 6 Hz, 8-$C(Me)_2$), 1.52 (3H, *s*, 1-CH_3), 1.00-2.6 (H-3, H-4, H-8, H-9, H-10), 4.77 (2H, *d*, 5-CH_2), 5.24 (1H, *dd*, J = 16.4, 8 Hz, H-7), 5.82 (1H, *d*, J = 16.4 Hz, H-6), 5.0 - 5.5 (1H, *br*, H-2); MS *m/z* (rel. int.): 204 $[M]^+$ (32.1), 161 (100), 133 (32.1), 119 (56.6), 105 (94.3), 91 (83), 81 (64.1), 79 (58.5), 77 (45.3), 67 (28.3), 55 (37.7), 41 (66).

α-Copaene (3)

Light yellow oily liquid; RT 9.05 min; ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.78 (3H, *s*, 1-CH_3), 0.85 (6H, *d*, J = 5.7 Hz, 8-$C(CH_3)_2$), 1.68 (3H, *s*, 1-CH_3), 5.20 (1H, *br*, H-4); MS *m/z* (rel. int.) 204 $[M]^+$ (20.6), 161 (91.6), 119 (99.1), 105 (100), 93 (48.6), 91 (42.9), 81 (28), 77 (22.4) 55 (20.6), 41 (33.5).

d-Limonene (4)

Colourless oily liquid; RT 4.25 min; $[\alpha]^{20}_{D}$: + 125.7°; IR ν_{max} cm^{-1}: 3080, 2930, 2855, 1640 1440, 1370, 880; ^{1} H NMR (90 MHz, $CDCl_3$): δ 1.67 (3H, *s*, H-10), 1.75 (3H, *s*, H-7), 1.2

2.10 (H-3, H-4, H-5, H-6), 4.69 (2H, *s*, H-9), 5.39 (1H, *br*, H-2); ^{13}C NMR (22.5 MHz, $CDCl_3$): See Table 11.

Ipsdienone (5)

Light yellow oily liquid; RT 5.83 min; UV λ_{max} (EtOH) nm: 225, 286; ^1H NMR (90 MHz, $CDCl_3$): δ 1.88 (3H, *s*, 2-CH_3), 2.14 (3H, *s*, H-1), 3.28 (2H, *s*, H-5), 5.09 (1H, *dd*, J = 8.3, 1.6 Hz, H-8_a), 5.15 (1H, *dd*, J = 14.6, 1.6 Hz, H-8_b), 5.25 (2H, *s*, 6-CH_2), 6.15 (1H, *br*, H-3), 6.41 (1H, *dd*, J = 14.6, 8.3 Hz, H-7); ^{13}C NMR (22.5 MHz, $CDCl_3$): See Table 13; MS *m/z* (rel. int.): 150 $[M]^+$ (3.2), 83 (100), 69 (8.6), 55 (29).

Citral b (7)

Light yellow oily liquid; RT 7.10 min; ^1H NMR (90 MHz, $CDCl_3$): δ 1.60 (3H, *s*, H-8), 1.69 (3H, *s*, H-9), 1.98 (3H, *s*, 3-CH_3), 2.10 - 2.80 (H-4, H-5), 5.11 (1H, *br*, H-6), 5.87 (1H, *d*, J = 8.6 Hz, H-2), 9.89 (1H, *d*, J = 8.6 Hz, H-1).

Citral a (8)

Light yellow oily liquid; RT 7.58 min; ^1H NMR (90 MHz, $CDCl_3$): δ 1.60 (3H, *s*, H-8), 1.69 (3H, *s*, H-9), 2.18 (3H, *s*, 3-CH_3), 2.10 - 2.40 (H-4, H-5), 5.03 (1H, *br*, H-6), 5.87 (1H, *d*, J = 8.3 Hz, H-2), 9.98 (1H, *d*, J = 8.3 Hz, H-1).

Perillaldehyde (9)

Light yellow oily liquid; RT 7.86 min; $[\alpha]^{21}_D$: + 113.4°; UV λ_{max} (EtOH) nm: 228; IR ν_{max} cm^{-1}: 3080, 2960, 2915, 2870, 2830, 2730, 1690, 1640, 1440, 1380, 890; ^1H NMR (90 MHz $CDCl_3$): δ 1.55 (3H, *s*, H-10), 1.60-2.50 (H-3, H-4, H-5, H-6), 4.55 (2H, H-9), 6.64 (1H, *br* H-2), 9.24 (1H, *s*, H-7); ^{13}C NMR (22.5 MHz, $CDCl_3$): See Table 14.

Piperitenone (10)

Light yellow oily liquid; RT 8.73 min; UV λ_{max} (EtOH) nm: 242, 269; IR ν_{max} cm^{-1}: 3020, 2980, 2930, 2850, 1660, 1620, 1420, 1380, 1300, 1220, 910, 870, 730; ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.81 (3H, *s*, H-10), 1.88 (3H, *s*, H-9), 2.04 (3H, *s*, H-7), 2.24 (2H, H-5), 2.63 (2H, H-6), 5.84 (1H, *br*, H-2); ^{13}C NMR (22.5 MHz, $CDCl_3$): See Table 15.

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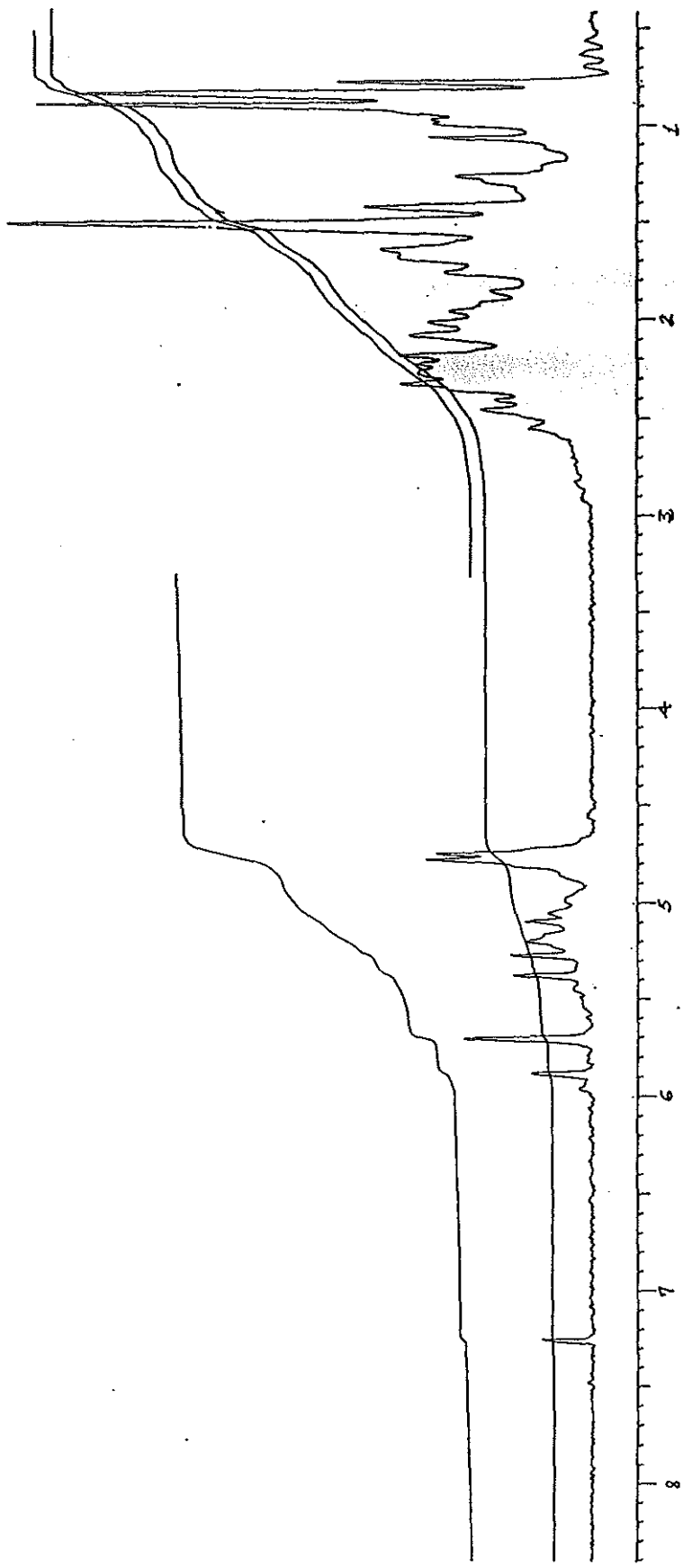
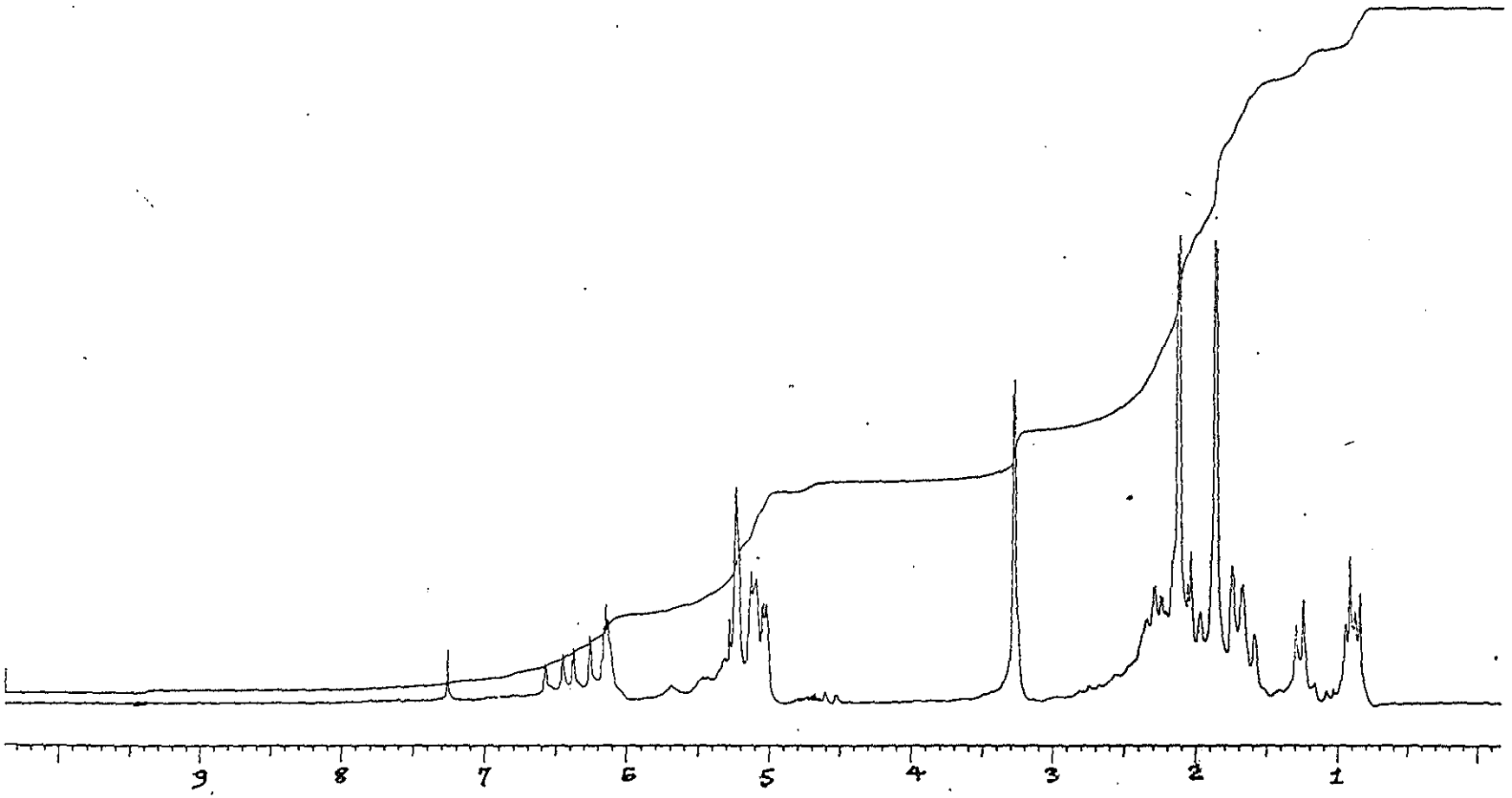
APPENDIX

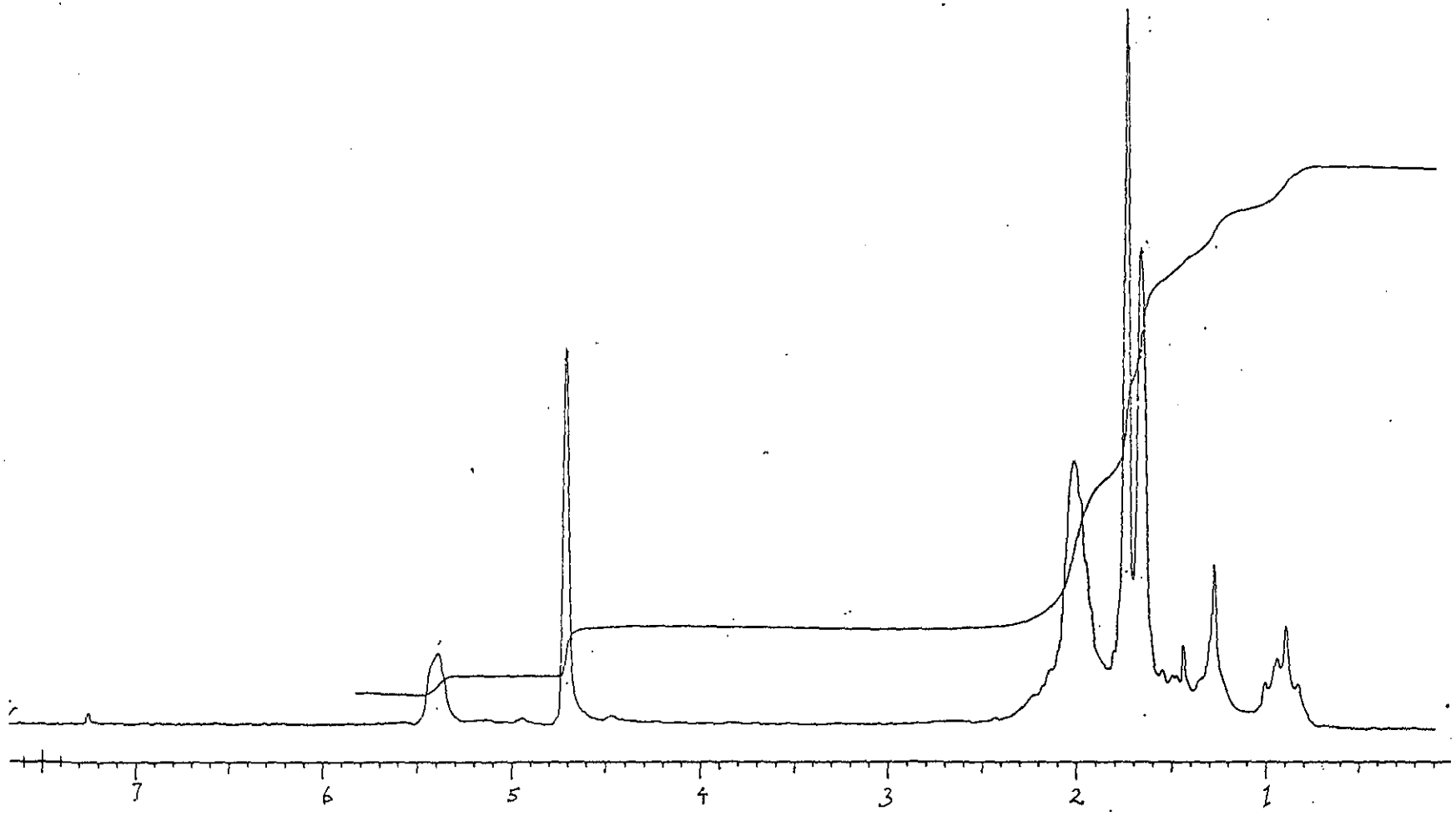


^1H NMR spectrum of Germacrene D

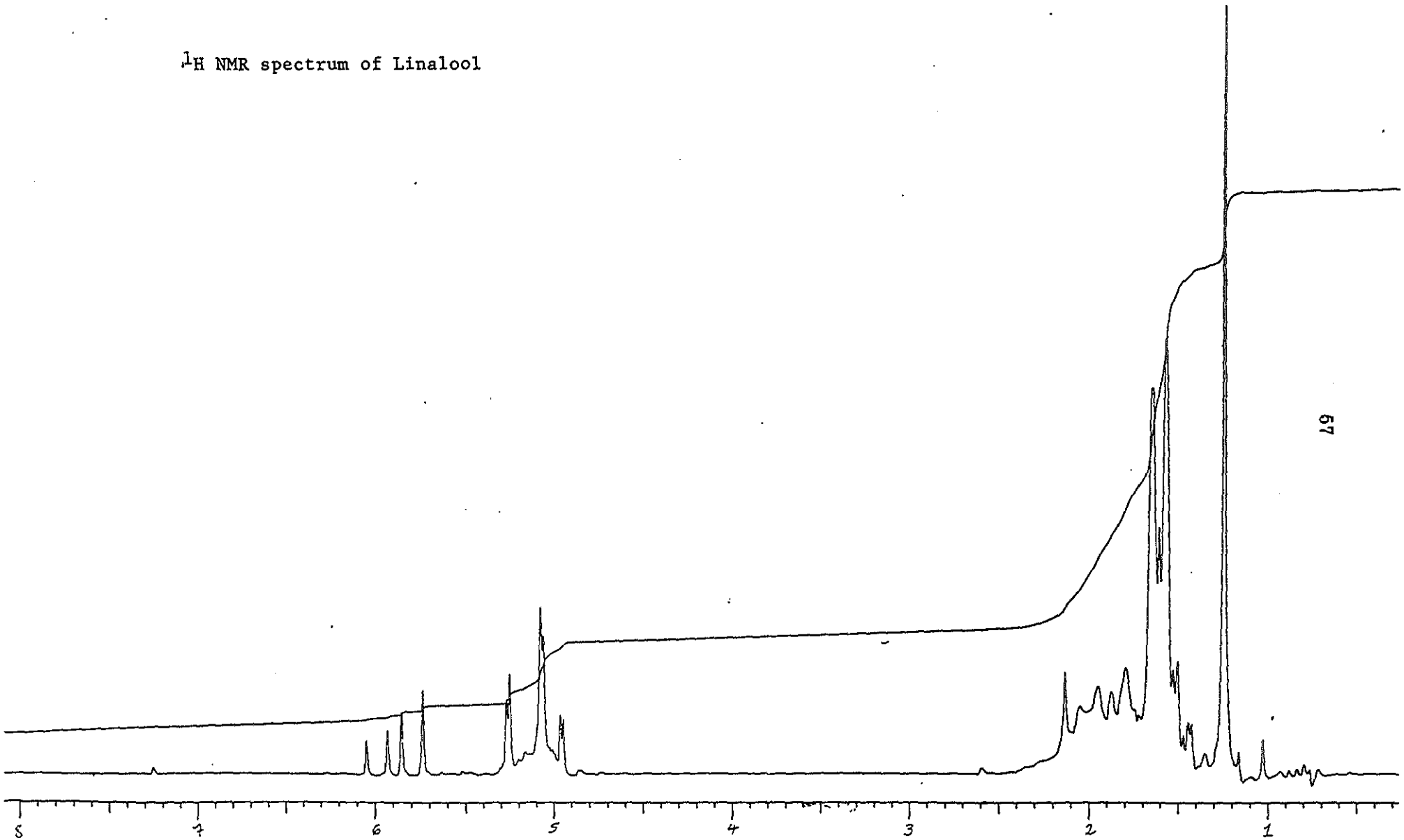
^{1}H NMR spectrum of Ipsdienone



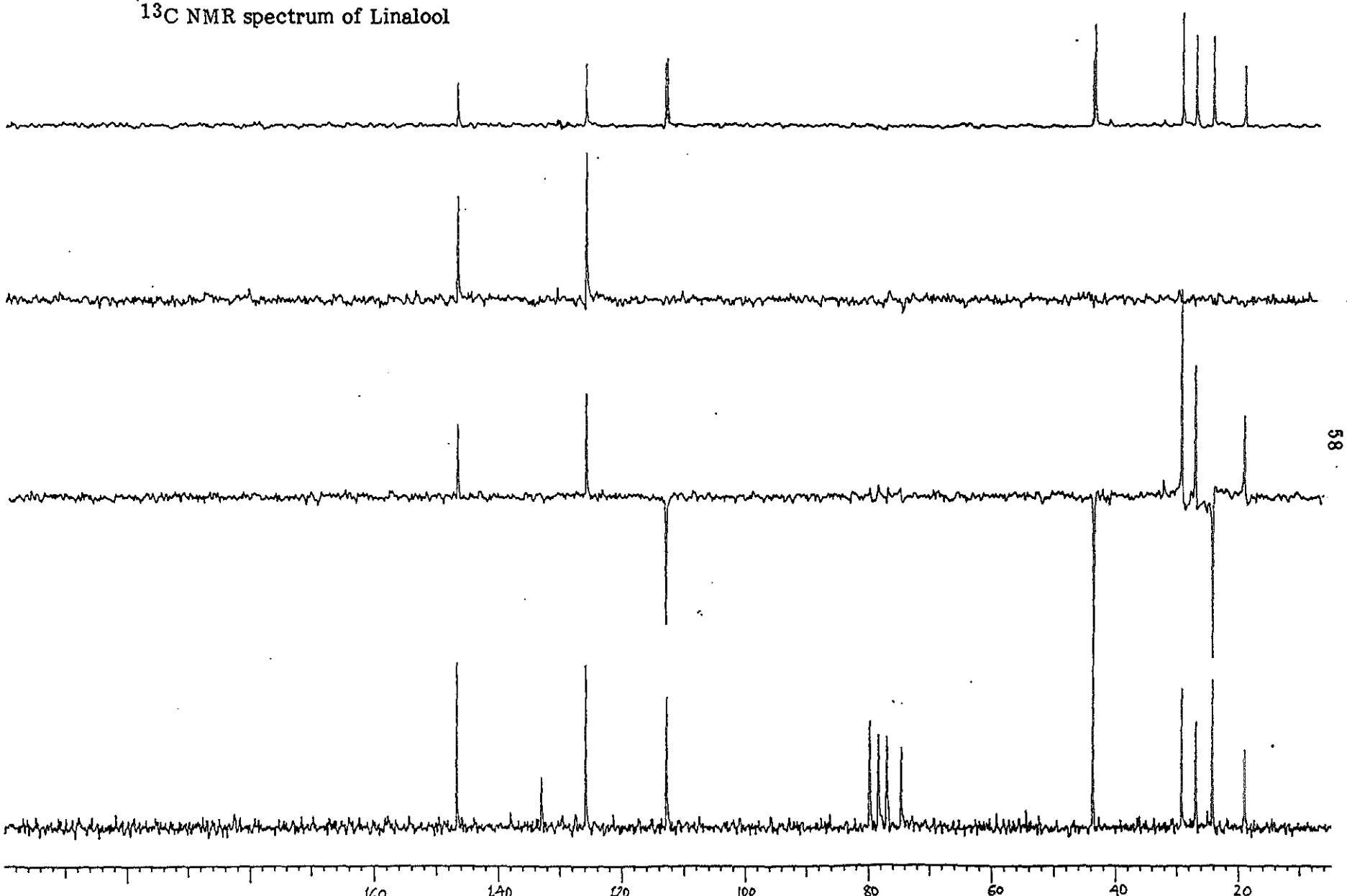
^{1}H NMR spectrum of Limonene



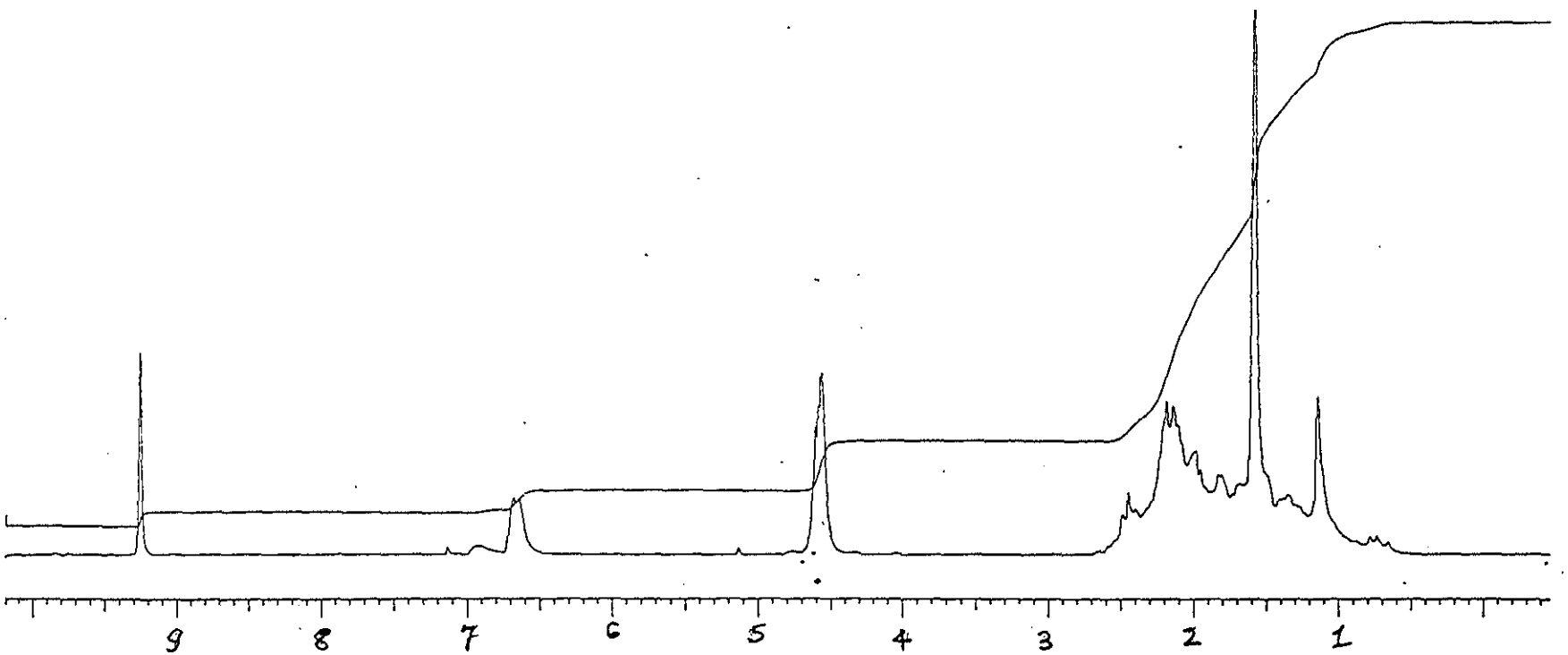
^{1}H NMR spectrum of Linalool



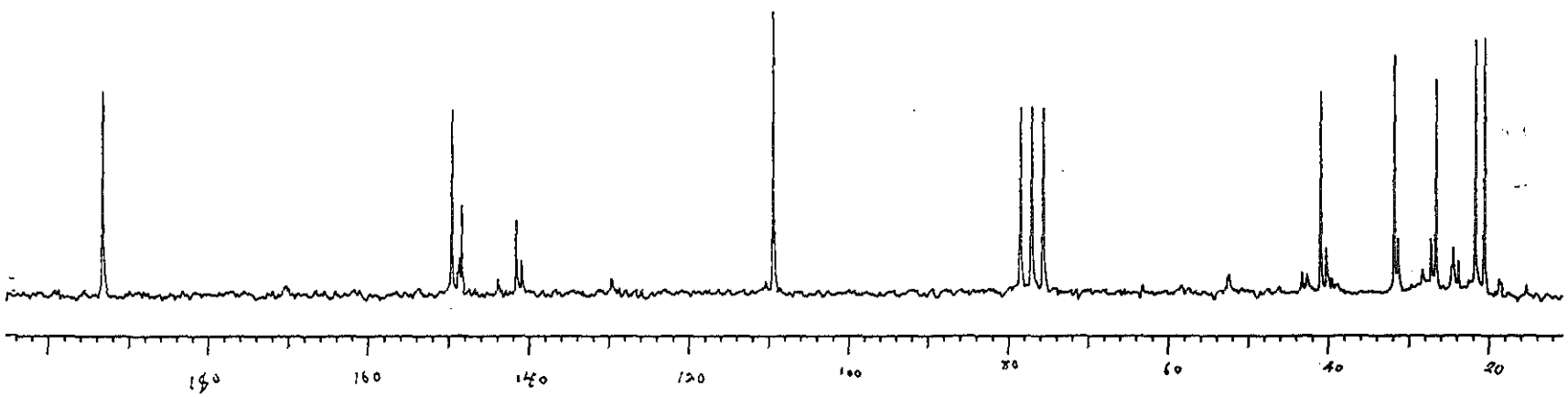
^{13}C NMR spectrum of Linalool

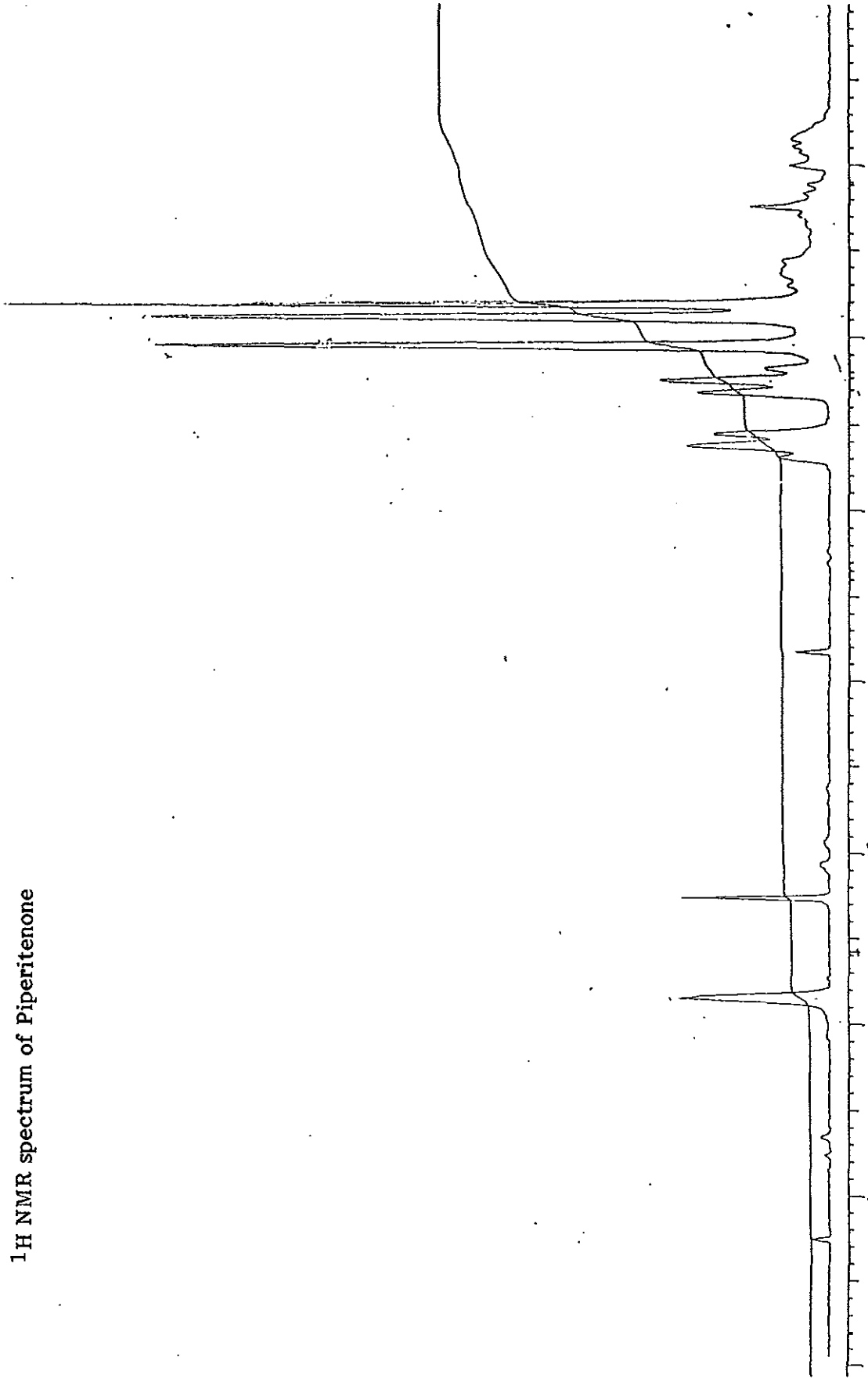


^{1}H NMR spectrum of Perillaldehyde



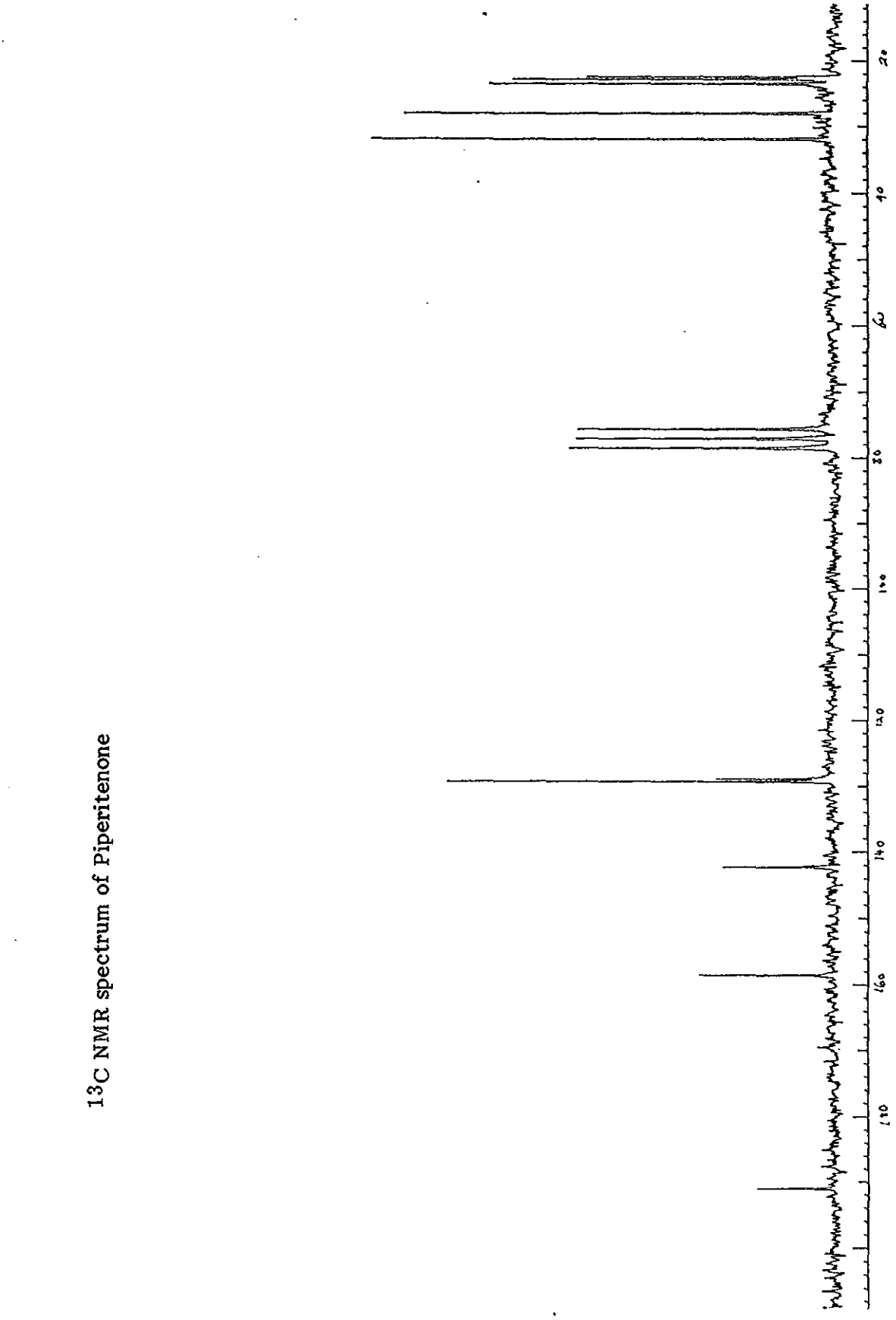
^{13}C NMR spectrum of Perillaldehyde





^{1}H NMR spectrum of Piperitenone

^{13}C NMR spectrum of Piperitenone



DECLARATION

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

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Date: June, 1992

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