ANALYSES OF THE ESSENTIAL OILS OF ARTEMISIA REHAN AND CYMBOPOGON CITRATUS

A Thesis
Presented to
the School of Graduate Studies
Addis Ababa University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science in Chemistry

by
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June 1980

To my father, G.Yohannes Andom
and my brother, Tsehaye G.Yohannes

ACKNOWLEDGMENT

I would like to express my deepest gratitude to my research advisor, Dr. Berhanu Abegaz, for his expert guidance. I would also like to thank Dr. Tesfaye Biftu for his interest and advice in the research.

Dr. Stan Evans of Finnigan (Hemel Hempstead) for running mass spectra on some of our samples, Dr. Jurgen Liebscher of Dresden University of Technology for providing standard samples, members of the department of chemistry (AAU) who showed interest in this work, and Ato Yilma Tamiru for typing this thesis are gratefully acknowledged.

Financial support from the Swedish Agency for Research Cooperation with the Developing Countries (SAREC) obtained through the Ethiopian Science and Technology Commission and from the Addis Ababa University which was used to cover the expenses incurred in the research work undertaken and in the preparation of this dissertation is gratefully acknowledged.

ABSTRACT

ANALYSES OF THE ESSENTIAL OILS OF
ARTEMISIA REHAN AND CYMBOPON CITRATUS

by
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Research advisor: Dr. Berhanu Abtaz

Artemisia rehan Chiov and Cymopogon citratus (DC) Stapf are widely grown in the highlands of Ethiopia. These plants occupy a significant position in Ethiopian plantlore.

The oil of Artemisia rehan, an endemic plant to Ethiopia, has been analysed for the first time. Eighteen components of the oil have been identified using GLC and/or MS. Two compounds, chamazulene and davanone were isolated from the oil and characterized using ir, nmr and ms.

Davanone (44%) and Camphor (27%) were found to be the major constituents of the oil.

Cymbopogon citratus which is also widely grown in the tropics, is used as a crop plant in many countries of the Western Hemisphere. The oil of the Ethiopian variety was analysed for the first time. Fifteen components of the oil have been identified using GLC and/or MS. The major component identified was geraniol (39%). The second major component (12%), not identified, is presumed to be an aromatic compound.

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1. INTRODUCTION

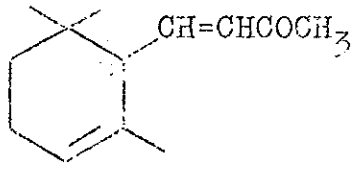
"Essential oils" or "etherial oils" are volatile oils obtained by the steam distillation of plants. This definition makes a distinction between the fatty oils (fixed oils) and the oils which are volatile. The principal constituents of essential oils are the terpenes, mainly hydrocarbons and their oxygenated derivatives. Some essential oils contain large amounts of non-terpene components. For example, methyl-salicylate makes up about 90% of anise oil and eugenol as much as 95% of clove oil.[1-4]

Essential oils have varied industrial applications. Because of their odor and high volatility, they are extensively used in the manufacture of perfumes, toilet soaps, sachetes, as flavoring materials, as essences for candy and ice-cream, and for flavoring alcoholic and non-alcoholic beverages. Still others have therapeutic, antiseptic, or bactericidal properties and so are valuable in medicine and dentistry. Some of the oils are used as clearing agents in histology; as solvents in the paint and varnish industries; as insecticides and deodorants; in the manufacture of various synthetic odors and flavors; and in such widely diversified products as chewing gums, tobacco, shoe polish, printer's ink, tooth paste, and fish glue[5]. In Ethiopia a variety of odoriferous plants are available which are used in some medications and as spices and condiments.

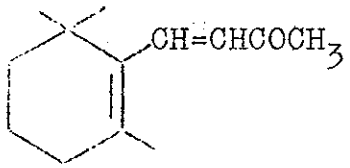
This study deals with, two plants, Cymbopogon citratus (D.C) Stapf; commonly known as lemongrass and Artemisia rehan Chiov. Both plants occupy a significant position in Ethiopian plantlore. The composition of the oil of A. rehan, an

endemic plant to Ethiopia is analyzed for the first time. Lemon-grass is widely grown in the tropics and is reported to have varying composition depending on its place of origin. The oil of the Ethiopian variety of C. citratus is analyzed for the first time and compared with reported composition of other varieties.

Lemon-grass, due to its high economic importance has now been successfully introduced as a crop plant in many countries including the Western Hemisphere, and large quantities of the oils are exported from Guatemala, Haiti, Brazil, Salvador, and other Latin American countries. The oil is known for its high citral content which is extensively used in perfumes, bath salts, cosmetics, and toilet soaps and as a flavoring substnace. It is also the source of substances like α-ionones (1) and β-ionones (2)



1



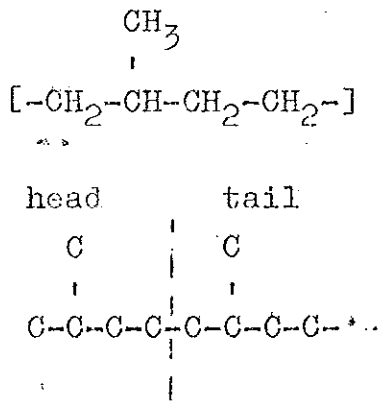
2

One of the ionones (β-ionone) is necessary in the synthesis of vitamin A; another (α-ionone) is the

raw material from which synthetic violet is made.[5]

1.1 Classification of terpenes

The word "terpene" is used to describe a compound which is a constituent of an essential oil and which contains carbon and hydrogen (and in some cases oxygen), and is not aromatic in character. The great majority of terpenes have carbon skeletons which can be built up, theoretically, by the union of two or more isoprene or isopentane residues. These units are usually united in a "head-to-tail" manner. This theory is referred to as the 'isoprene rule'.



A 'head tail' arrangement of isopentane skeleton.

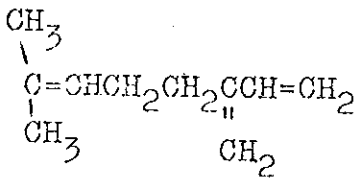
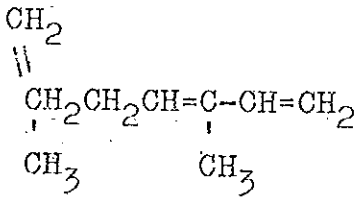
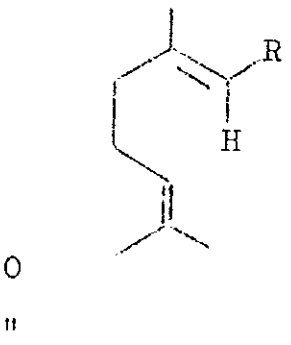
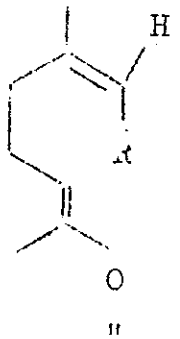
The main classification of terpenes is on the basis of the number of isoprene units in the molecule, a single 'terpene unit' being equal to two 'isoprene units'.[6]

Table 1. Classification of Terpenes

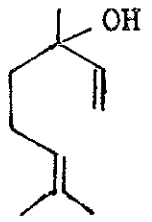
Number of isoprene units	Number of carbon atoms	Group
2	10	Monoterpene
3	15	Sesquiterpene
4	20	Diterpene
5	25	Sesterpene
6	30	Triterpene
8	40	Tetraterpene
> 8	> 40	Polyterpene

The monoterpenes are further classified into acyclic, monocyclic and bicyclic terpenes. The bicyclic terpenes are subdivided into five subdivisions. The sesquiterpene group is also classified into acyclic, monocyclic, bicyclic and tricyclic. These classes and subclass are shown in the table below along with representative examples.[6,7]

Table 2. Classes and Subclasses of Monoterpenes and Sesquiterpenes.

Class	Examples
1. Monoterpene	
a. acyclic	
-non-oxygenated	
	3
	Myrcene
	
	4
	Ocimene
- oxygenated	
	
	5. R=-C-H, Geranial (Citral a)
	6. R=-CH_2OH, Geraniol
	
	7. R=-C-H Neral (Citral b)
	8. R=CH_2OH Nerol

../..



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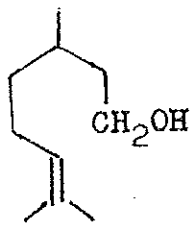
Linalool



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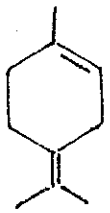
Citronellol

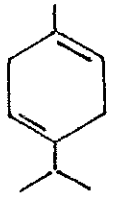
b. Monocyclic

- non-oxygenated



11

Terpinolene



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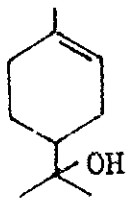
γ-Terpinene

- oxygenated



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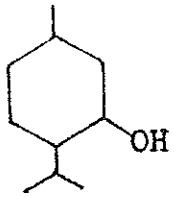
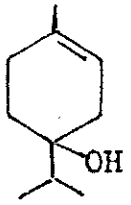
α-Terpineol



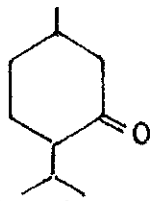
14

Menthol



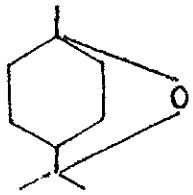
15

Terpinen-4-ol



16

Menthone



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1,8-Cineole

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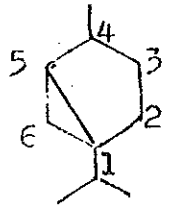
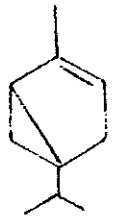
c. Bicyclic

(i)



Thujane type

-non-oxygenated



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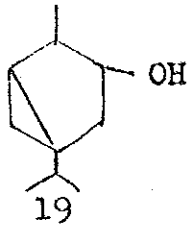
α-Thujene

-oxygenated



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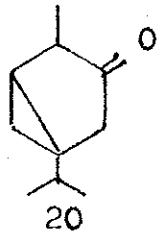
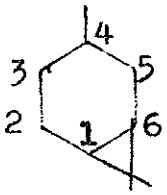
Thujyl alcohol



20

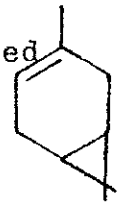
Thujone

(ii)



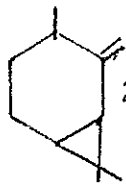
Carane type

-non-oxygenated



21. Car-3-ene

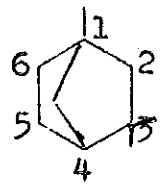
-oxygenated



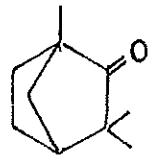
22. Carone

../..

(v)



Fenchane type



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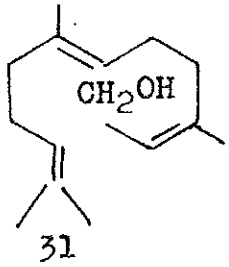
Fenchone

II. Sesquiterpene

(i) acyclic



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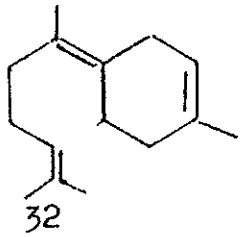
Farnesol

(ii) monocyclic



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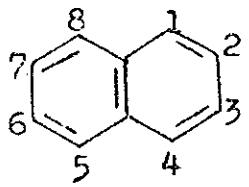
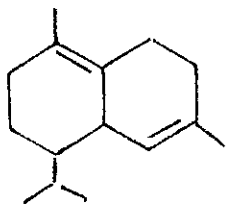
Bisabolene

(iii) Bicyclic



a. Naphthalene type

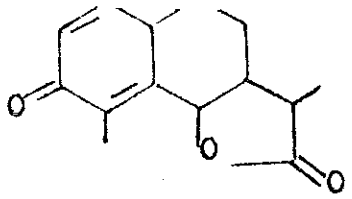
-non-oxygenated



33

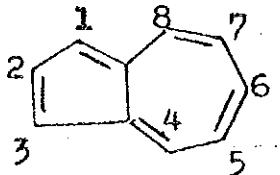
Cadinene

-oxygenated

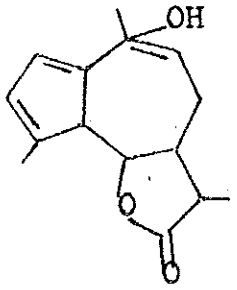


34

Santonin

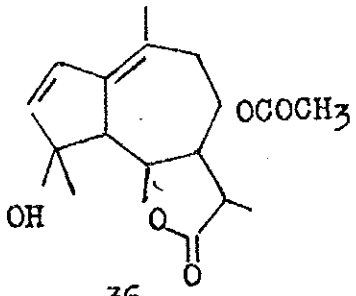


b. Azulene type



35

Artabsin



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Matricin

1.2 Biogenesis of Monoterpenes and Sesquiterpenes

It is believed that the isopentane unit, so important in terpene chemistry, may be derived from three molecules of acetic acid (37). The mechanisms involved in the biogenesis of terpenes could be envisaged as follows:[8]

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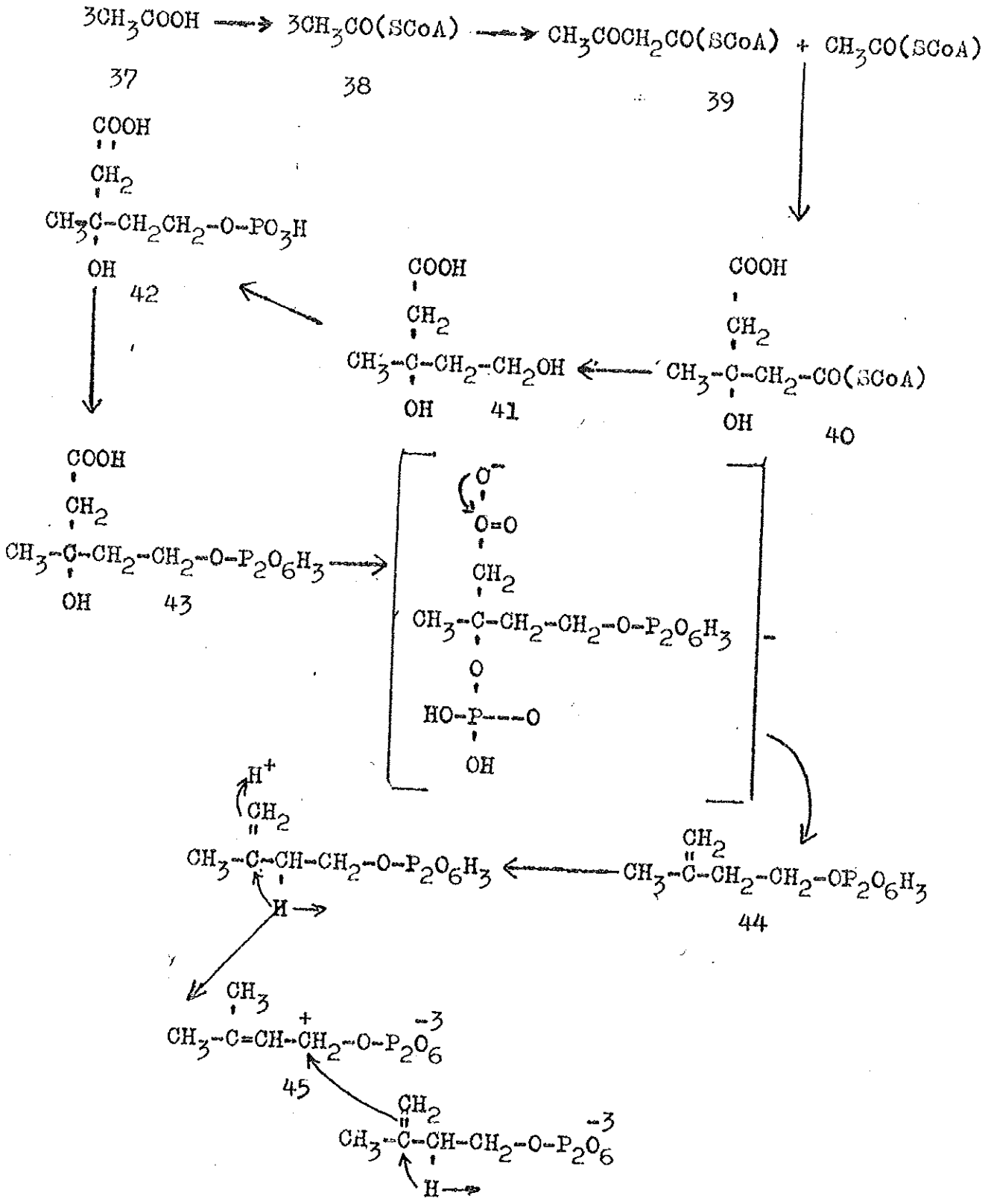
Condensation of acetyl CoA (38), obtained from acetic acid, with acetoacetyl CoA (39) yields β-hydroxy-β-methyl glutaryl CoA (40) Figure 1. The latter on reduction of the thiolester group yields mevalonic acid (41). The monophosphate ester of β-hydroxy-β-methyl glutaryl CoA, 5-phosphomevalonic acid (42) with adenosine triphosphate yields another ester, mevalonic acid -5-pyrophosphate (43). Isopentyl pyrophosphate (44) obtained subsequently isomerizes to give the pyrophosphate ester of dimethylallyl alcohol (45). The allylic nature of dimethylallyl pyrophosphate permits it to act as an alkylating agent so that condensation with a second molecule of isopentyl pyrophosphate occurs to form farnesylpyrophosphate (47). The following figure shows the mechanisms involved in the biogenesis of terpenes. The cyclization and subsequent steps ignored here, are indicated later in this section.

Figure 1. Biogenesis of Monoterpenes and Sesquiterpenes



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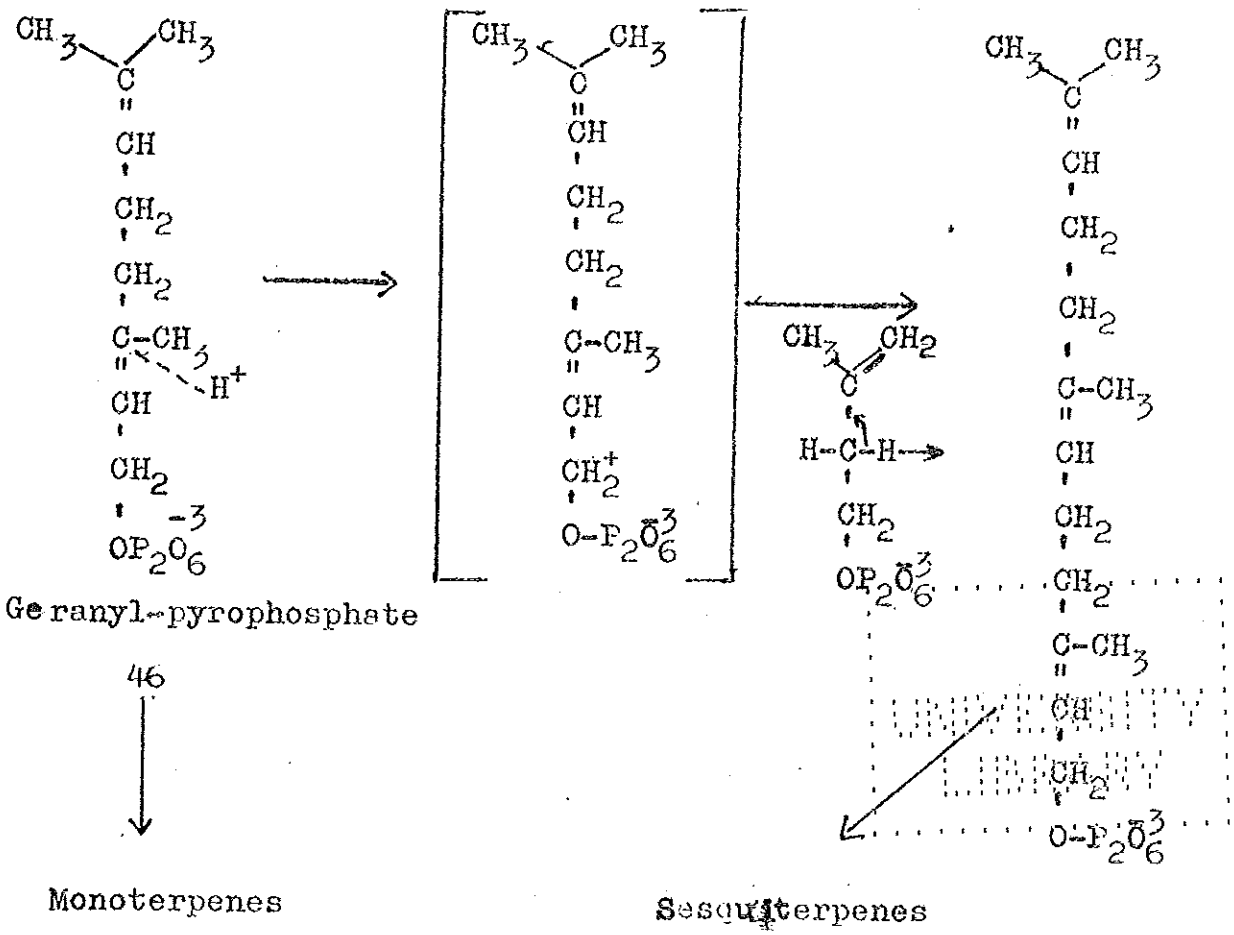
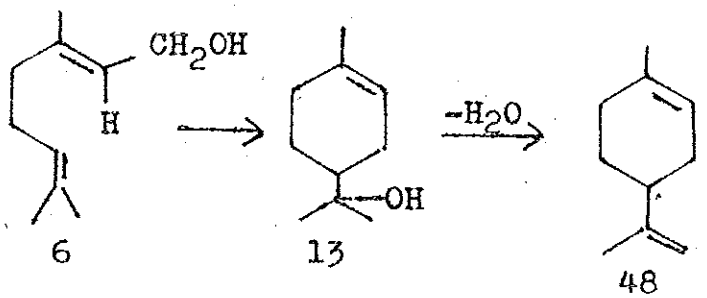


The acyclic monoterpenes obtained from geranyl-pyrophosphate (46) give rise to monocyclic monoterpenes by cyclization routes? Geraniol (6) for example, may give rise to α-terpineol (13) and to limonene (48)



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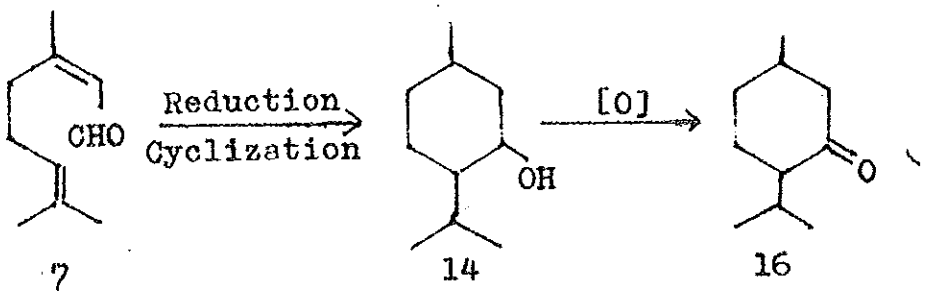
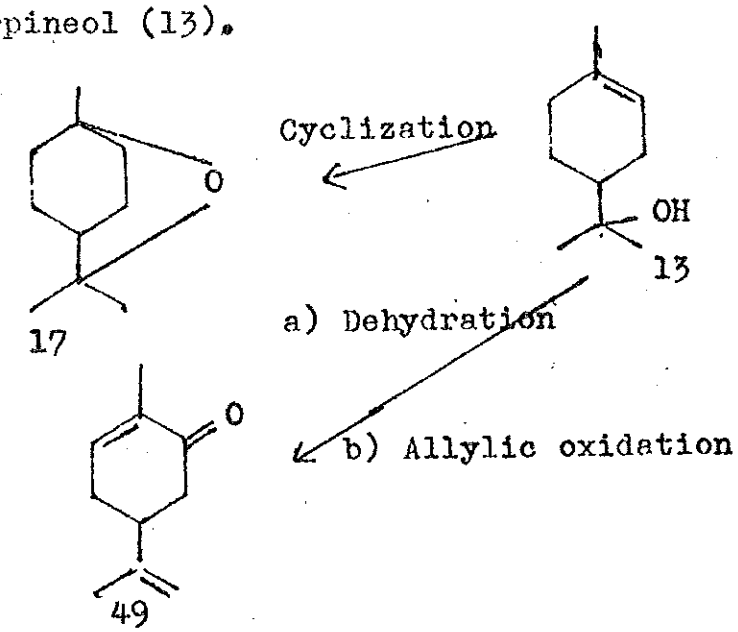
Citral b (7) on reductive cyclization, could afford menthol (14) which in turn upon oxidation would yield menthone (16)



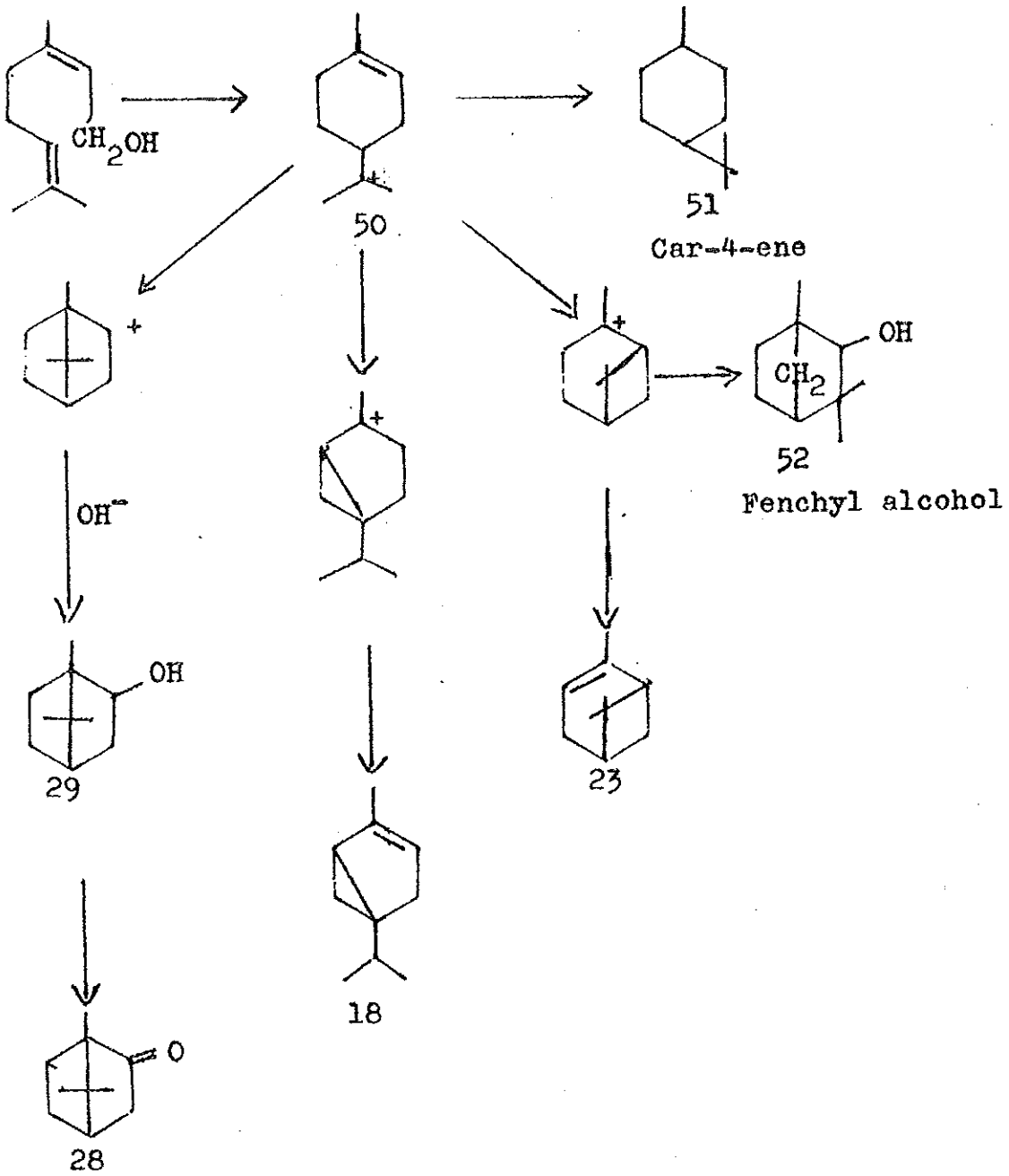
Cineol (17) and carvone (49) may originate from α-terpineol (13).



Bicyclic monoterpenes may also arise from cyclic precursors. Geraniol (6), for example, may be the starting material for the biosynthesis of most members of this group. Ruzicka[10] has proposed that these transformations occur via an intermediate

carbocation (50) (Scheme I)

Scheme I



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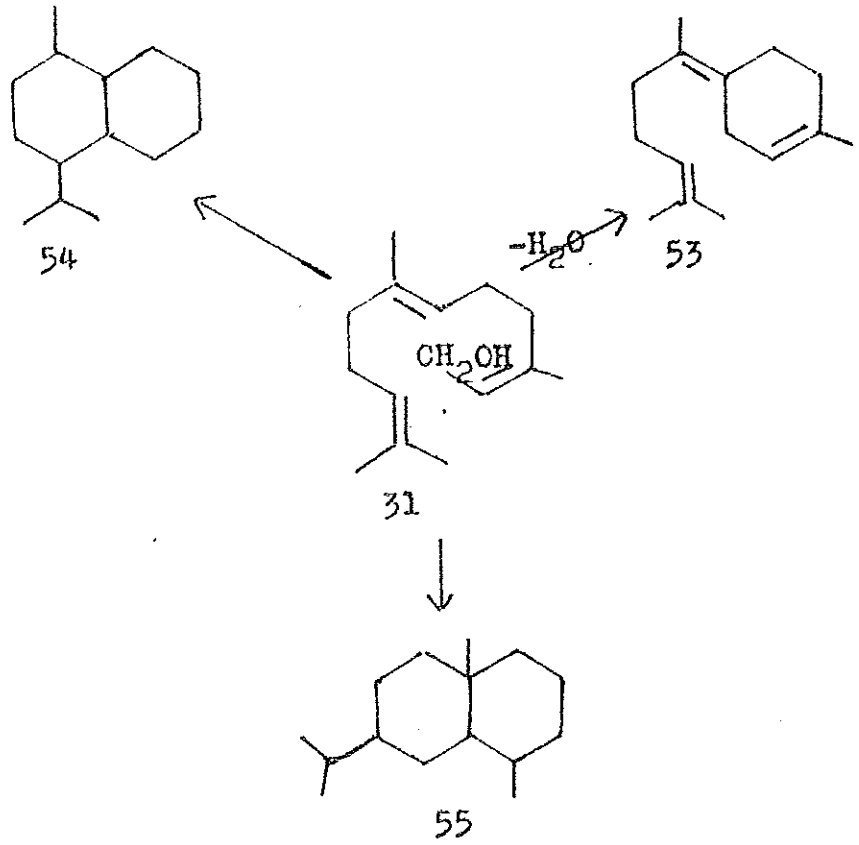
In the case of sesquiterpenes, farnesol follows analogous lines to that of geraniol (Scheme I). Three possible modes of cyclization of farnesol (31) (as farnesyl pyrophosphate in figure 1) in vivo give rise to three main types of cyclic sesquiterpenes, i.e bisabolene type (53), cadinene type (54) and eudesmol type (55). (Scheme II).

Scheme II



The azulenes and related compounds and other larger ring sesquiterpenes are also derivable from farnesol:

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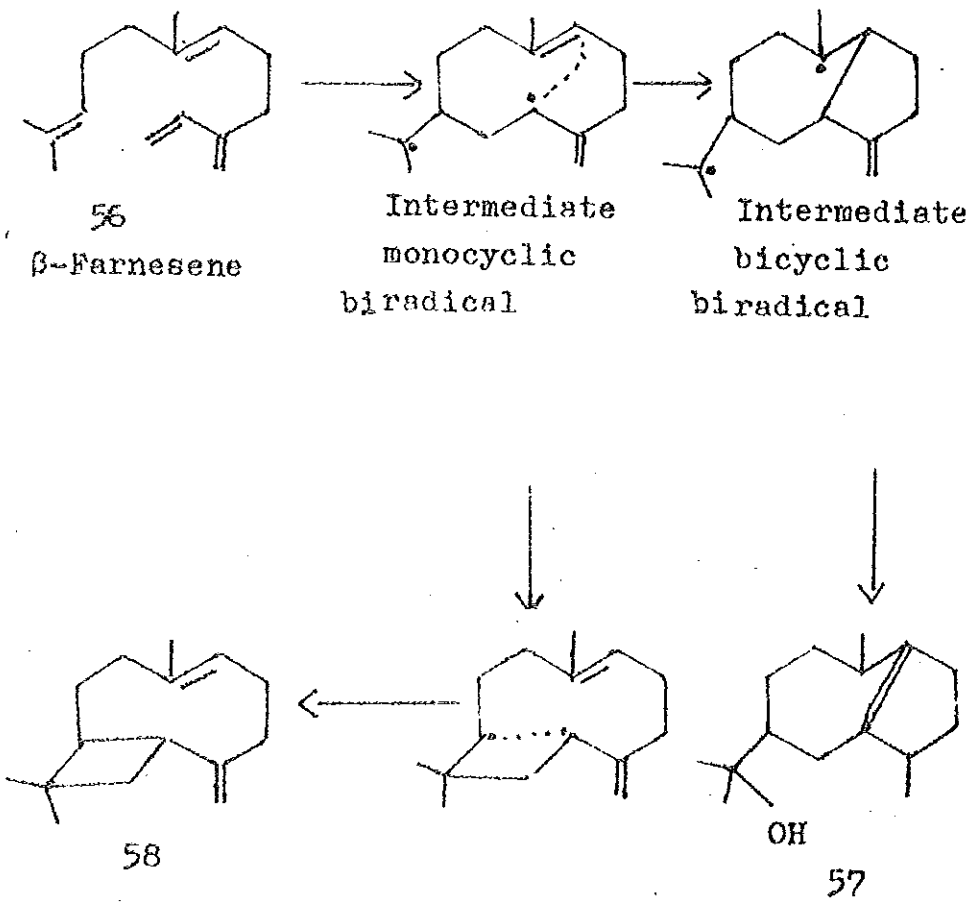
guaiol (57) and caryophyllene (58) for example, may arise as follows (Scheme III).

Scheme III

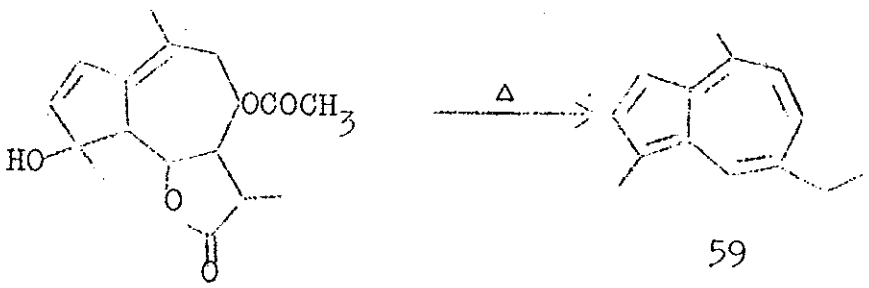


1.3 Isolation of Essential Oils

Essential oils are most often separated from different parts of plants by steem distillation at

atmospheric pressure. The oils are insoluble in water and can easily be separated. Most of the flavor and aromatic components are found in the oil layer, little being present in the water. In cases where the steam distillate contains low quantities of essential oil, the latter can be separated from the aqueous layer by extraction with hexane or light petroleum ether.

Some essential oil constituents are heat-labile, and structural changes can take place during isolation by steam distillation at atmospheric pressure. For example, chamazulene (59), which was isolated from a dark blue colored oil of Artemisia daruttii was reported[11] to be obtained as the result of dehydration, decarboxylation and deacetylation of a sesquiterpene lactone matricin (36)



36 59

In such cases steam distillation at reduced pressure or other methods of isolation are employed.

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In some plants, for example, in fruits, the oils are found mainly in ductless glands located on the outer layer of the peel. They are usually obtained by cold press methods. These oils may be extracted also with volatile organic solvents at low temperatures. Petroleum ether is sometimes used to extract thermolabile compounds. Other solvents like benzene and acetone are sometimes used but these solvents extract materials other than essential oils.[12]

1.4 Characterization of Essential Oils

Gas liquid chromatograph (GLC) is an excellent tool used in the identification of the constituents of essential oils. Prior to GLC analysis, the hydrocarbons and oxygenated components of the oil can be separated by liquid-solid chromatography (LSC) on silicic acid or alumina. The oil is applied in a hexane solution to a column containing alumina or silica gel. Hydrocarbons are eluted with light petroleum ether. On successive elution with ethylacetate the oxygenated monoterpenes and the sesquiterpenes are obtained. Alternatively, preparative GLC can be used to separate essential oil constituents on non-polar packings. The monoterpene hydrocarbons generally having higher vapor pressures than the oxygenated components are eluted first. However, several low boiling oxygenated components are also removed with the monoterpene hydrocarbons. The sesquiterpene hydrocarbons are eluted after the oxygenated monoterpenes, since their boiling

points in general are higher than those of the oxygenated derivatives. The eluates can be collected and analyzed by any suitable method.[12]

Another less common method developed by Clark and Bernhard,[13] referred to as the slurry method, is also employed to separate the hydrocarbons from the oxygenated compounds. Silicic acid is added to the essential oil disolved in hexane or pentane and allowed to stand with occasional swirling for a period of time, after which the solvent containing the hydrocarbon fraction is filtered off leaving the oxygenated components adsorbed on the silicic acid. These oxygenated compounds are then extracted from the adsorbent with ethylacetate.

The fractions obtained via the aforementioned methods are then subjected to GLC analysis. In GLC analysis, retention time is characteristic for a compound and depends upon its solubility in the sationary phase. It would, therefore, be reasonable to infer that one could identify the components of a mixture by direct comparison of their retention time data with those obtained for standard compounds. Retention time, however, varies with column temperature, column length, and the type of stationary phase and carrier gas flow rate. Therefore, a more reliable correlation can be obtained by the use of relative retention time data using internal or external standards. Identification on the basis of relative retention time data does not however constitute unequivocal proof of identity. The observed peak could be that of another compound which by coincidence has the

same retention time or it can be a composite peak of two or more components having the same or almost the same retention times and together giving the appearance of a single peak. Thus, care should be taken in analysis of essential oils. Therefore, the use of adding a small amounts of the expected pure compounds to the mixture and observing an increase in the area of the peak at the expected retention time coupled with the use of varying column temperatures and use of different columns solves such identification problems.[14]

A still better method of analysing essential oils is by using a gas chromatograph interfaced with a mass spectrometer (GLS/MS). Here compounds are identified on the basis of molecular weights, fragmentation pattern and retention times. More recent methods employed are GLC and GLC/MS with data processing devices. Thus such computerized GLC instruments perform all integration and calculation tasks and generate chromatograms and such data as computed areas, retention times etc.

The GLC/MS fitted with a computer can not only process acquired data but can also compare it with stored information (mass spectra of known samples). It is, therefore, capable of giving a more accurate information regarding the identity of a compound.[15]

2. Oils of Artemisia

The genus, Artemisia is one the largest and most widely distributed genera. This genus with nearly 300 species, is found predominantly in the northern temperate regions of the world.[16]

Early investigations[17] on these plants were carried out in search of an anthelminthic drug, santonin (34), which was believed to be present only in Artemisia species. As a consequence of such investigations, several reports[18-57] regarding the essential oil in particular the sesquiterpene lactones appear in the literature.

A summary of the constituents of the most common species of Artemisia oils from the literature is given in table 3. The sesquiterpene lactones found in these plants is also given in table 4.

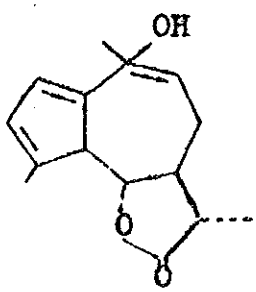
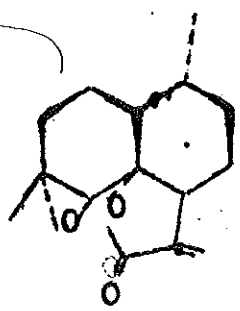
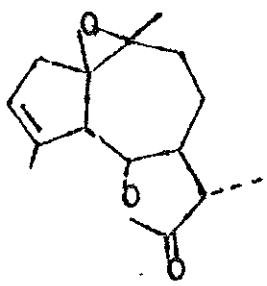
Table 5. Components of Different Artemisia species

	Species	Artemisia ketone (a)	Borneol (b)	Cadinene (c)	Camphene (d)	Camphor (e)	Carvone (f)	Caryphyllene (g)	Cineole (h)	Cuminaldehyde (i)	p-Cymene (j)	Eugenol (k)	Geranylacetate (l)	Isovaleric acid (m)	Limonene (n)	Methyl-heptenone (o)	Myrcene (p)	Pinene (q)	Sabinene (r)	Terpineol (s)	Thujone (t)	Reference (u)
1	A. absinthium		+		+	+		+	+		+				+			+			+	18
2	A. afra		+		+	+			+									+				19, 20
3	A. annua			+	+			+	+									+				21
4	A. arborescens		22%																			22
5	A. austriaca					+			+		+											23
6	A. brevifolia				+	+			+		+										+	24
7	A. capillaris	+		+					+		+			+	+		+	+				24
8	A. copa													+								25
9	A. eucina								+		+							+			+	26
10	A. feddei	+			+			+	+		+							+	+		+	27
11	A. fergamensis		+			+	+		+										+			28
12	A. grata				+		+															29
13	A. japonica	+		+	+			+							+						+	24

	Species	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)	(n)	(o)	(p)	(q)	(r)	(s)	(t)	(u)
14	A. kaschgarica					+																30
15	A. kurramensis					20%															63%	31
16	A. lagocephala		+			+																32
17	A. lessingiana					+																33
18	A. leucodes					88%			+													34
19	A. macrosiadia					35%			46%													35
20	A. maritima					+			+	+	+	+						+	+		+	36
21	A. mendozana					+																37
22	A. mogoltavica					17%			12%													38
23	A. mongolorum					+			+		+						+	+				38
24	A. obtusiloba										+						+	+				38
25	A. persica		+		+													+				33
26	A. porrecta		+			+			+					+							+	39
27	A. porcera					+			+		+	0.2%						+	+			40
28	A. santolinfolia		+			+			+													41
29	A. scoparia			+					+			+				+		+				42
30	A. terrae-albe						+		+						+							43
31	A. vulgaris								+						+							44

+ present

Table 4 Sesquiterpene Lactone Isolated from the Genus Artemisia

Species	Compound	References
A. absinthium	 35 Artabsin	45
A. annua	 60 Aretannuin	46
A. arborescens	 61 Arborescin	47

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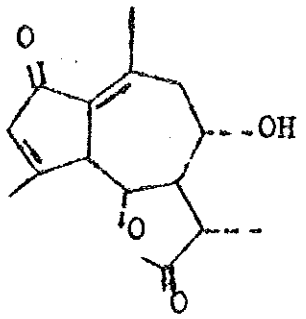
4. A. austriaca



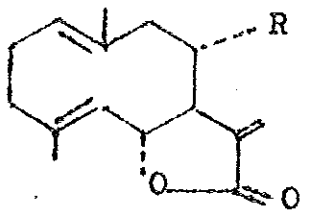
48 , 49

62

Deacetylmatricarin

5. A. balchanorum

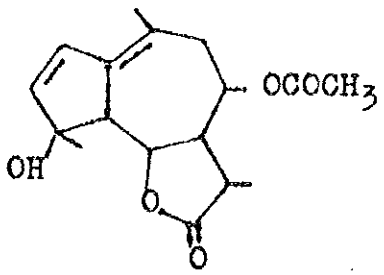
50, 51



63

Costunolide

6. A. carutti



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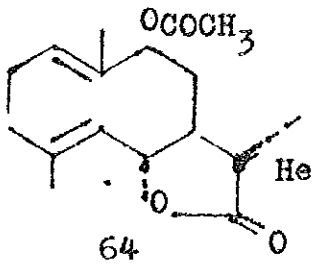
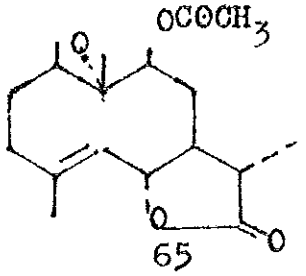
7. A. herba alba



52

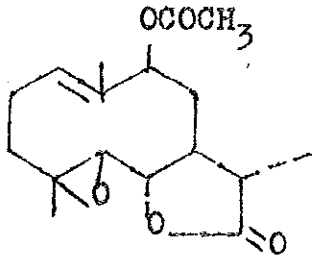
Herbolide A

64



65

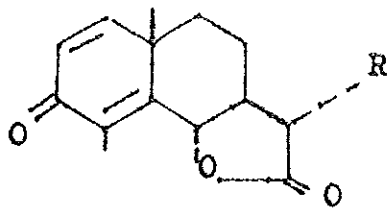
Herbolide B



66

Herbolide C

8. A. kurramensis



53, 54, 55

67. R=α-Me, α-Santonin
68. R=β-Me, β-Santonin

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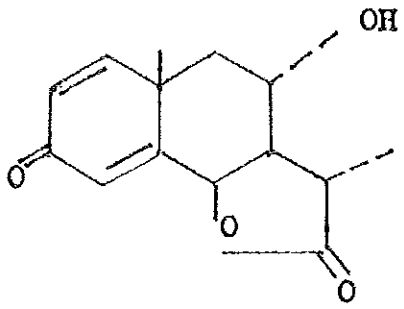
9. A. maritima



56, 57

69

Artemsin

3. Oils of Cymbopogon

The different species of Cymbopogon have been investigated for the major content in the oils since 1907.[58-73] In most cases it is indicated that the different species contain citral, geraniol, geranylacetate, monoterpene hydrocarbons like limonene, camphene and pinene and sesquiterpenes.

Early investigations were done mainly by chemicals means[16-20, 22, 29] and only the major components were reported. Recent advances in gas chromatography, mass spectrometry and other instrumental methods have opened the possibility for analysing minor components in essential oils in a way that was not possible by chemical means before. It appears now that many of the essential oils of Cymbopogon have been analyzed for their content of citral and geraniol, the presence of which determines the commercial value of the plant. However, oils of Cymbopogon species of different localities differ in their composition. Such diversifications is clearly observed in the following tables 5 and 6.

Table 5. Components of Essential Oils of Different Species of Cymbopogon

	Plant	Borneol	Camphene	Citral	Elemicine	Geraniol	Geranyl acetate	Limonene	Δ'menthenone	Pinene	Piperitone	Terpineol	Thujene	Reference
1.	C. colaratus			40%		23%	10%							58
2.	C. martini (motia)*					73-84%								59
3.	C. martini					90%								64
4.	C. martini(sofia)*					40-30%								59
5.	C. nardus	+	+					+		+		+	+	60
6.	C. procerus				35%									61
7.	C. sennarensis							+	+					62
8.	C. sennarensis**										44-77%			63

*There are two varieties of C. martini, namely, motia and sofia.

**Plant from Eritrea northern province of Ethiopia.

+amount not specified in the literature

Table 6. Physical Property and Components of *C. citratus* Oil from Different Countries

	Source	Physical Property	% Volatile oil	Citral	Dipentene	Geraniol	Linalool	Methyl heptenol	Methyl heptenone	Myrcene	Nerol	Reference
1.	Brazil	d_{25} 0.8730-0.8816 n_{20} 1.4851-1.4879 α (-0.25)-(-0.48°)		77-79%								65
2.	Ceylon	d_{15} 0.895-0.908 α 1-5° n_{20} 1.483-1.489		66-85%	+	+	+	+	0.2-0.3%	10-20%	+	66
3.	Ghana	-	2.1 - 2.5%	70-78%								67
4.	Phillipines		0.3-0.48%	31-45%								68
5.	Sicillian	d_{15} 0.8969-0.8856 $[\alpha]_D$ 0.85-1.6 n_D^{20} 1.4865-1.4825		+		+						69
6.	Somaliland	-		80%	3-4%				+	12%		70
7.	Siam	d_4^{26} 0.8741 $[\alpha]_D^{26}$ 0.36 n_D^{26} 1.487		63%								71
8.	Vietnam & India			80-90%								72-73

Table 7. Identified Components and Percentage Composition of the Oil of A. rehan using 10% Carbowax and 10% Ucon columns on Chromasorb WHP 80/100 and 100/120 mesh respectively.

	Substance	Retention time (sec.) Carbowax	Ucon	Percentage Composition
1.	α-Pinene	192	157	0.04
2.	Campene	240	157	0.01
3.	β-Pinene	300	192	0.33
4.	1,8 Cineole	564	132	0.54
5.	γ-Terpinene	624	288	0.65
6.	Menthone	1176	756	0.10
7.	Camphor	1392	816	26.92
8.	Menthol	1560	1200	0.63

The chromatograms obtained by running the oil on 10% Ucon, 10% carbowax and capillary column coated with Carbowax 20M showed 19 and 25 components in the first two columns respectively and more than 80 components in the latter. The oil, thus, was run on column of silica gel and sucessively eluted with hexane and ethylacetate to separate the hydrocarbons from the oxygenated compounds. The oil as well as the fractions collected were analyzed on GLC/MS. Identification was done by comparison of the mass spectra of the components of the oil with spectra of standard samples that were run on GLC/MS as well as with spectral data in the literature.[74]

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Table 8. GLC/MS Analyses of Components of Oil of *A. rehan* on 10% Carbowax 20M on Chromosorb WHP 100/120 mesh.

	Compound	Mass to charege ratio (M^+/e)	Retention time (Sec.)
1.	-	136	105
2.	Myrcene	136	165
3.	Linalool	154	225
4.	Camphor	152	690
5.	Terpinen-4-ol	154	750
6.	-	204	795
7.	-	234	1200
8.	Davanone	236	1530

Similarly, the oil and the fractions obtained from column chromatography were analyzed using GLC/MS with data system.

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Table 9. Analyses of Components of Oil of A. rehan on 50m Capillary Column Coated with Carbowax 20M using GLC/MS with Data System.

	Compound	M^+/e	Retention time (sec.)	Percentage Composition
1.	Bornyl acetate	196	502	1.76
2.	Camphor	152	422	
3.	Davanone	236	1016	44.30
4.	Ethylcinnamate	176	1100	2.93
5.	Eudalene	184	1479	2.05
6.	Eudesmol	222	1220	0.81
7.	α-Terpineol	154	502	5.16

The non-terpene compounds identified by this method are bornylacetate (70), ethylcinnamate (71) and eudalene (72). The first two compounds have been identified as components of some other essential oils. Eudalene, however, has not been reported as a natural product. It is reported[6] that eudesmol (73) on dehydrogenation with selenium or sulphur at high temperature yields eudalene. These are severe conditions and cannot be comparable to the isolation and chromatographic conditions that the oil was subjected to.

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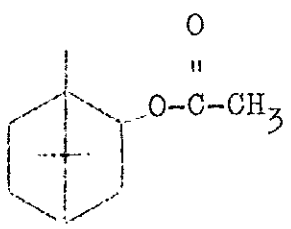

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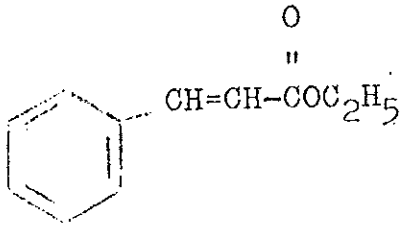
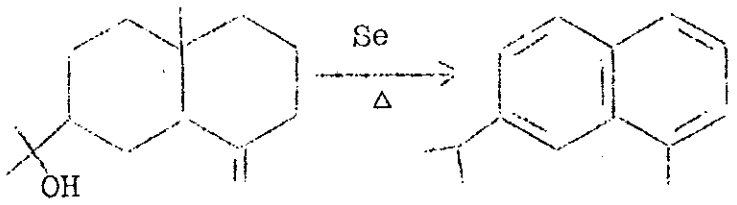


71



73 72

Thus, eventhough, eudesmol is also identified in the oil, it is unlikely that eudalene arose from it. It is, however, possible that another more labile and highly functionalized precursor may have undergone dehydration and/ or decarboxylation to yield eudalene. It would be interesting to confirm if indeed such a precursor exists in the plant and determine its structure.

In the analyses of A. rehan oil by GLC/MS two components were noted to have the same molecular ion (M^+/e) of 176. This was somewhat

4. Results and Discussion

4.1 Artemisia rehan

The oil of this plant was obtained by steam distillation at atmospheric pressure. The oil which was obtained in 0.20% yield, (density 0.9123) has a characteristic blue color and a pleasant odor. The intense blue color precluded the measurement of optical rotation and reference index.

The analyses of the oil was carried out using mainly GLC and GLC/MS. Two columns were used in GLC analyses using isothermal and programmed oven temperatures. Identification of peaks were done mainly by comparison of the retention times with standard samples using the two columns. Some of the components that were not separated under isothermal conditions were resolved when run under programmed oven temperatures. Camphene and α-pinene, myrcene and β-pinene and camphor and menthone were not separated on Ucon liquid support. They were, however, separated on Carbowax column. Identification of the components 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. The table below shows the retention times of the identified components in the oil under programmed oven temperatures using both columns.

surprising since the common isomeric terpenoids do not have such molecular weight. Close examination of the mass spectra (see figures 2 & 3) further revealed not only identical fragmentation pattern but also similar ion intensities. Comparison of these spectra with stored spectra in the data system suggested the identity of these two components to be ethylcinnamate. The observation that these two compounds have identical mass spectra but different retention times can only be explained by assuming the two components to be geometrical isomers. The cis or trans arrangement of the groups about the double bond apparently does not alter the type of fragmentations occuring in the ion source of the mass spectrometer.

Taking into consideration the higher boiling point of the trans isomer, it is thought that the component with the higher retention time could be the trans form. It is infact the component which is present in larger amount (2.93%) while the other component amounts to 1.38%. Similar report appears in the literature[1] i.e the trans isomer is the one that is encountered in essential oils with high yield.

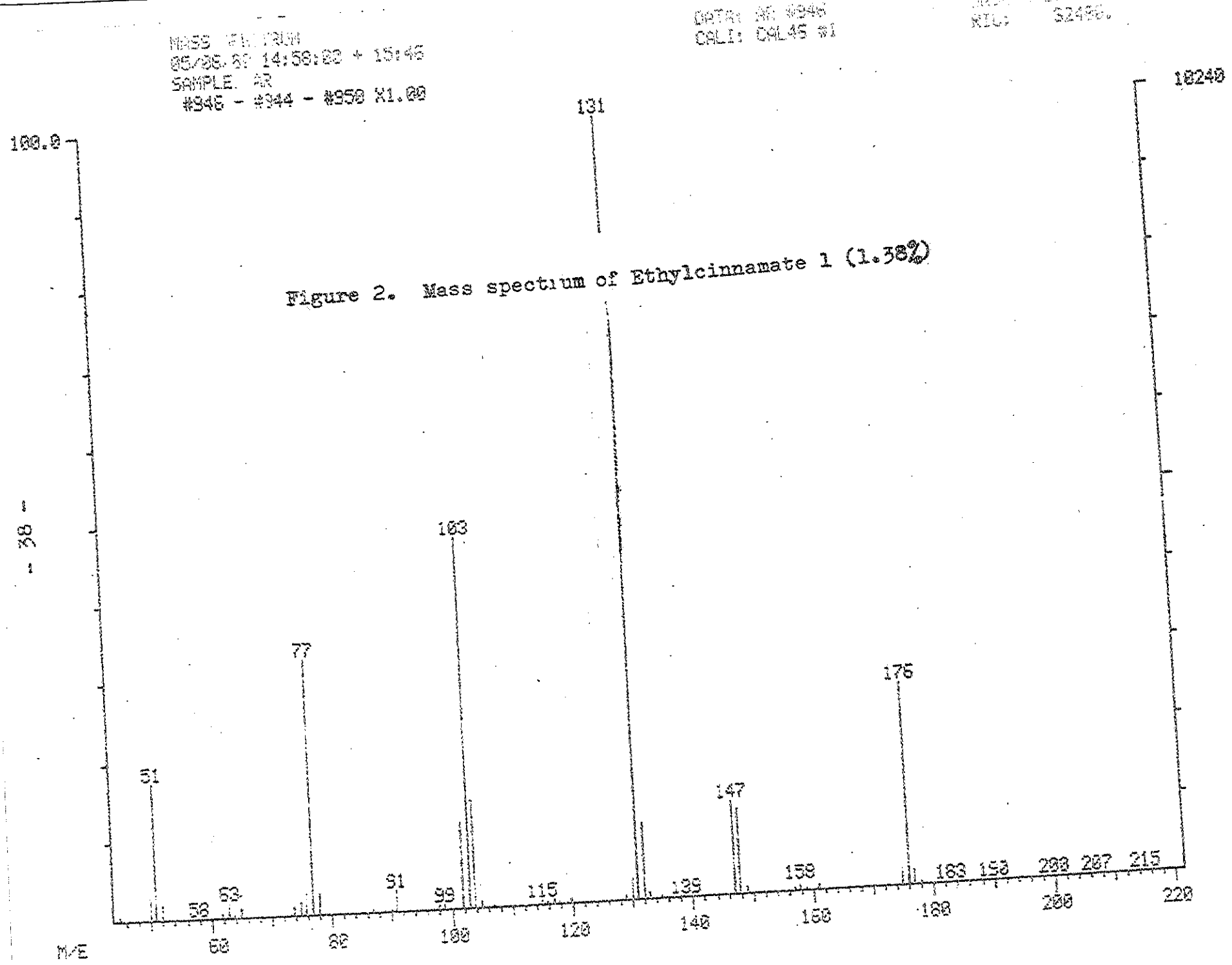


Figure 2. Mass spectrum of Ethylcinnamate 1 (1.38%)

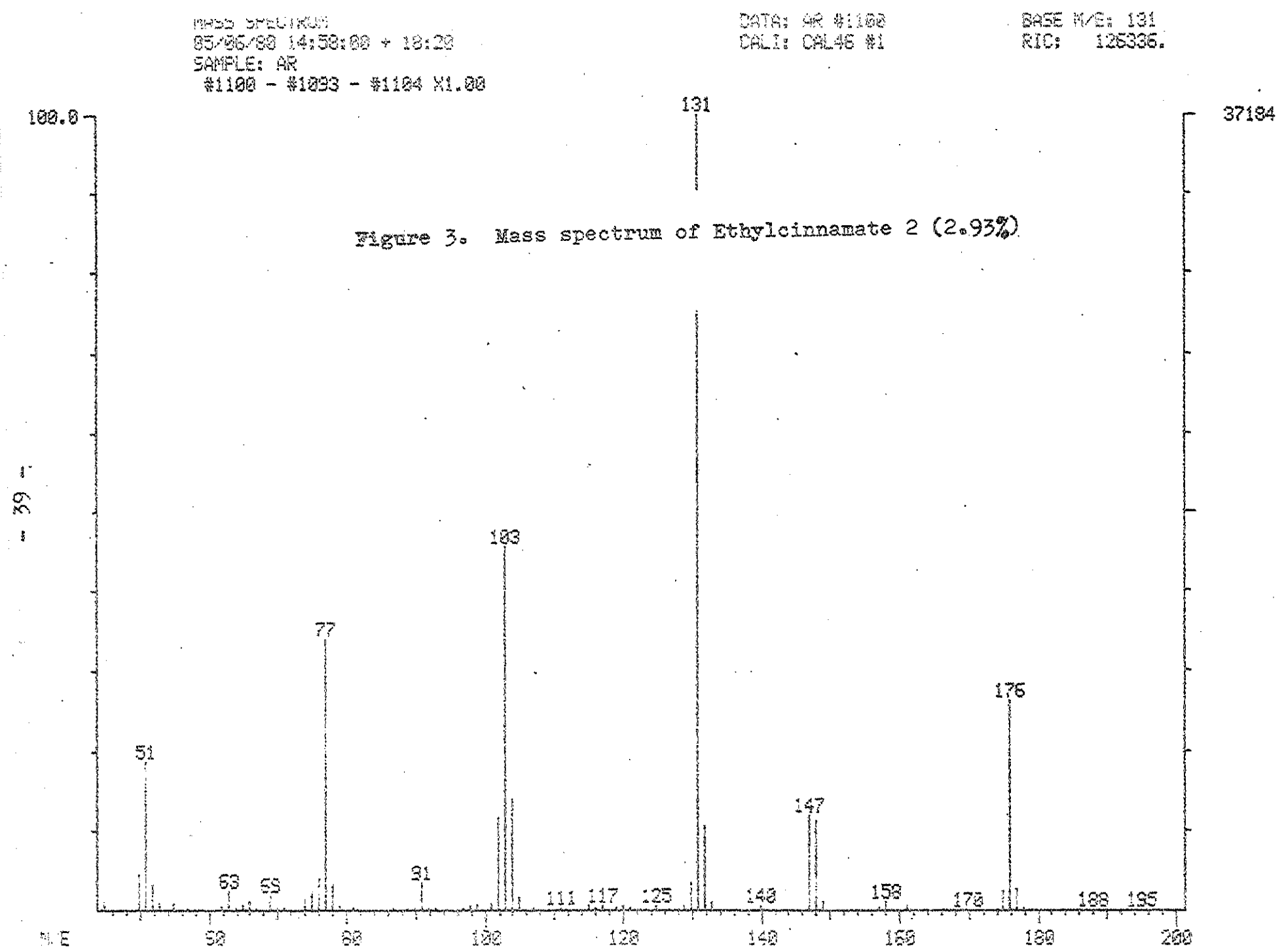
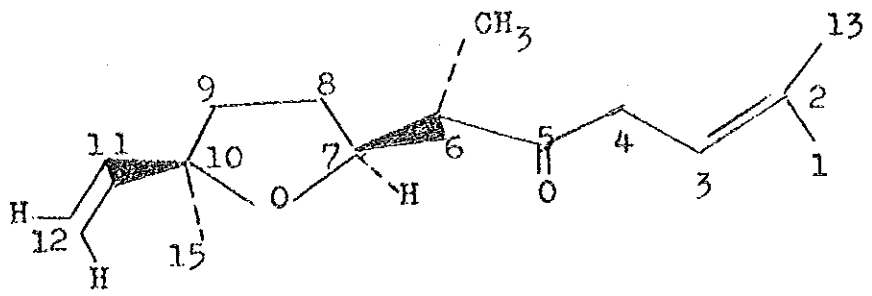


Figure 3. Mass spectrum of Ethylcinnamate 2 (2.93%)



74

Davanone (74) (M^+/e 236) was also found to appear at two different retention times on the chromatogram. This compound has been reported as the major component of the essential oil of A. pallens.[75] Thomas et al[76] have suggested the isomer of natural (+)-davanone to be 6S, 7S, 10R-2, 6, 10-trimethyl-7, 10-oxidododeca-2, 11-diene-5-one and no report appears in the literature regarding other isomers of this compound in essential oils. In addition, a complete study of the stereochemistry of davanone about the three chiral centers does not appear in the literature. Thus, further methods of analyses should be employed to identify the two isomers noted by our method of analysis.

4.1.1 Isolation and Characterization of Chamazulene

The blue oil obtained from the plant was run on a column of silica gel and eluted with hexane. The separation of the blue component on the silica gel was clearly

observed as a blue band separated from the other components of the oil. Different hexane eluents that contain the blue component gave a single spot on tlc. It gave a single peak on Ucon liquid support, but did not appear on Carbowax. The yield obtained after evaporation of hexane was 0.28% from the oil. The compound was identified as chamazulene on the basis of its nmr, ir, uv-vis, ir & ms data with literature,[77]

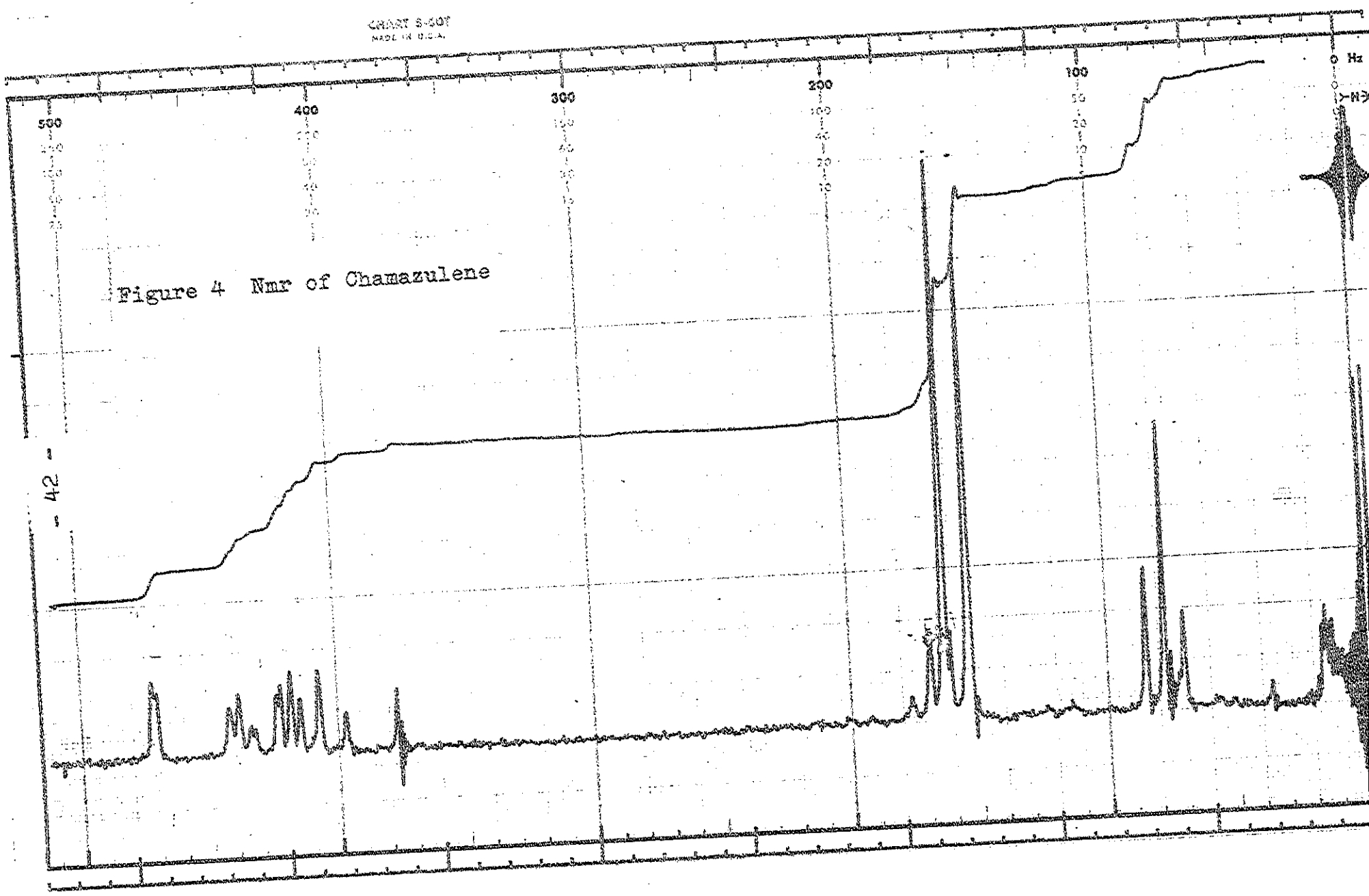


Figure 4 Nmr of Chamazulene

This blue component is most likely an artifact, obtained as the result of dehydration decarboxylation and/or deacetylation of a sesquiterpene lactone like artabsin (35) or matricin (36).

The formation of chamazulene (59) during steam distillation of other plant materials has been reported.[11,77] In search for the precurssors of chamazulene led to the isolation of artabsin and matricin which upon heating in slightly acidic media gave the blue compound chamazulene.

No attempt was made to determine if matricin, artabsin or any other precurssor is present in this plant.

4.1.2 Determination of Chamazulene in the Oil of A. rehan using Visible Spectroscopy

The isolated chamazulene was used in this study to determine the composition of chamazulene in the oil of A. rehan. Two different known concentrations in 95% ethanol were used to calculate the extinction coefficient from the absorbance of the oil at λ max (600nm) of chamazulene. The absorbance of the oil at λ max and the extinction coefficient

were then used to calculate the concentration of chamazulene in the oil. The value obtained was 0.26% from the oil. The amount of chamazulene obtained from gas chromatogram (0.24%) and from the isolated chamazulene by column (0.28%) appear to be in good agreement with the value obtained by this method.

4.1.3 Isolation and Characterization of Davanone

The oil of A. rehan after the blue component has been eluted with hexane from a column of silica gel gave a yellow oil upon successive elution with ethylacetate. This oil gave two major peaks and several other minor peaks on GLC. This fraction was further run on a column of silica gel with hexane - ethylacetate mixtures to give a fraction which exhibited to contain the major component (ca. 80%). This component was identified as davanone (74) from its nmr, ir and ms data with literature.[75]

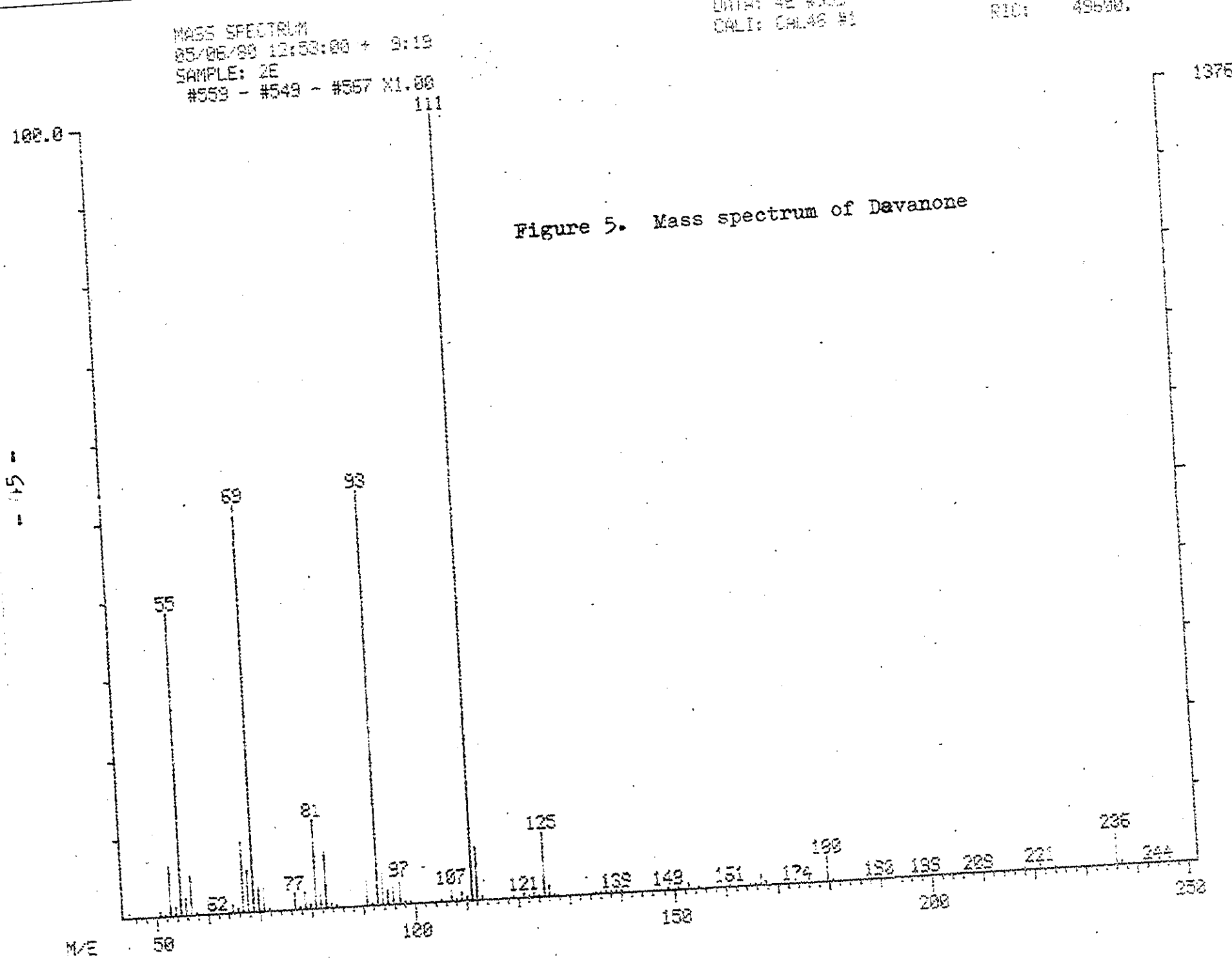


Figure 5. Mass spectrum of Devanone

Figure 9. NMR of Devarone

5. Cymbopogon citratus

The oil of this plant was obtained by steam-distillation at atmospheric pressure. The yield and physical property of the oil is given below.

Table 10. Yield and Physical Property of Oil of C. citratus.

		Physical Porperty at 20°C	
Yield	Density	Optical Rotation	Refractive Index
4.32%	0.9000	+9.1°	1.4814

The oil of this plant was chromatographed on a column of silica gel and eluted successively with hexane and ethylacetate. The fractions obtained in this manner as well as the oil were analysed on GLC and GLC/MS. The methods of identification of the components of the oil was done in the same manner as specified for A. rehan.

The results obtained by each method are summarized in the following tables.

Table 11. Identified Components and Percentage Composition of the Oil of C. citratus using 10% Ucon and 10% Carbowax 20M on Chromosorb WHP 80/100 and 100/120 mesh respectively.

		Retention time (sec.)		
	Substance	Carbowax	Ucon	Percentage Composition
1.	α-Pinene	192	157	0.13
2.	Camphene	240	157	0.05
3.	Myrcene	492	192	0.12
4.	Fenchone	1008	564	0.25
5.	Menthone	1452	756	0.12
6.	Menthol	1560	1200	0.49
7.	Borneol	2016	360	4.98
8.	Geraniol	2496	1752	39.17

Table 12. GLC/MS Analysis of Oil of C. citratus on 10% Carbowax 20M on Chromosorb WHP 100/120 mesh.

	Substance	M^+/e	Retention time (sec.)	Percentage Composition
1.	-	154	240	1.00
2.	-	170	630	4.87
3.	Citral	152	975	5.00
4.	Geraniol	154	1545	
5.	-	222	2160	3.00
6.	-	220	2490	12.00

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Table 13. Analysis of Oil of C. citratus on 50m Capillary Column Coated with Carbowax 20M using GLC/MS with Data System.

	Substance	M^+/e	Retention time (sec.)	Percentage Composition
1.	Δ^3 carene/ocmene+	136		
	Terpinolene+	136		
	Linalool	154	458	3.36
	itral b	152	630	3.28
	itral a	152	699	10.15
	Nerol	154	738	4.50
	Citronellol	156	746	5.24
	Geraniol	154	874	
	-	220	909	

+Analyzed by GLC/MS Finnigan model 3200F attached with Finnigan model 6100 data system using 50m capillary column coated with OV-17. The retention time and percentage composition is not given. The remaining compounds were run on, GLC/MS Finnigan model 4000 attached with Finnigan model 2000 Incos data system.

6. Summary

a. Artemisia rehan

No report has appeared in the literature regarding the oil of this plant. However, several works[19-44] done at the genus level indicate camphor (28) and 1,8-cineole (17) to be the most common components. In some of the oils of the species thujone (20) is major component which is not true in our case.

In the oil of A. rehan we have found davanone (74) and camphor (28) as the major components. In addition, it has been noted that there appears to exist two isomers of davanone from data obtained by GLC/MS which has not been reported before in other essential oils.

b. Cymbopogon citratus

The major components of the oils of this plant are geraniol (6) and presumably an aromatic compound with molecular ion peak of 220 (Figure 7). Previous reports[65-73] indicate citral as the major component (>50%) of lemon-grass oil. However, in two of the reports mentioned earlier,[59, 64] geraniol is indicated as the major component of C. martini.

The mass spectra of the compound with molecular ion of 220 was compared with mass spectra of sesquiterpenes such as santalol and cedranone in the literature[74] and showed no resemblance. The fragmentation pattern of this compound at low m^+/e also did not resemble the MS of the common terpenoid compounds (borneol, menthol, menthone,

terpineol, etc.).

The presence of intense molecular and other high mass ions in the spectrum strongly suggests that this component is most probably an aromatic compound.

The components of the two oils identified and the different methods employed is summarized below.

Table 14. Components of the Oil of A. rehan and C. citratus.

	Compound	M^+/e	A. rehan	C. citratus	Method of Identification
1.	Borneol	154		+	GLC
2.	Bornylacetate	196	+		MS^+
3.	Camphene	136	+		GLC
4.	Camphor	152	+	+	GLC
5.	Δ^3carene/ocimene	136		+	MS^+
6.	Chamazulene	184	+		MS,ir,nmr,uv-vis
7.	1,8 Cineole	154	+		GLC
8.	Citral a	152		+	MS^+
9.	Citral b	152		+	MS^+
10.	Citronellol	156		+	MS^+
11.	Davanone	236	+		MS,ir,nmr
12.	Ethylcinnamate	176	+		MS^+

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	Compound	M^+/e	A. rehan	C. citratus	Method of Identification
13.	Eudalene	184	+		MS^+
14.	Eudesmol	222	+		MS^+
15.	Fenchone	152		+	GLC
16.	Geraniol	154		+	GLC, MS^+
17.	Linalool	154	+	+	MS^+, MS
18.	Menthol	156	+	+	GLC, MS
19.	Menthone	154	+	+	GLC
20.	Myrcene	136	+	+	GLC, MS
21.	α-Pinene	136	+	+	GLC
22.	β-Pinene	136	+		GLC
23.	γ-Terpinene	136	+		GLC
24.	Terpinen-4-ol	154	+		MS
25.	α-Terpineol	154	+		MS^+
26.	Terpinolene	154		+	MS^+
27.	Nerol	154		+	MS^+
28.	-	220		+	MS^+, MS

$^+$MS with data system

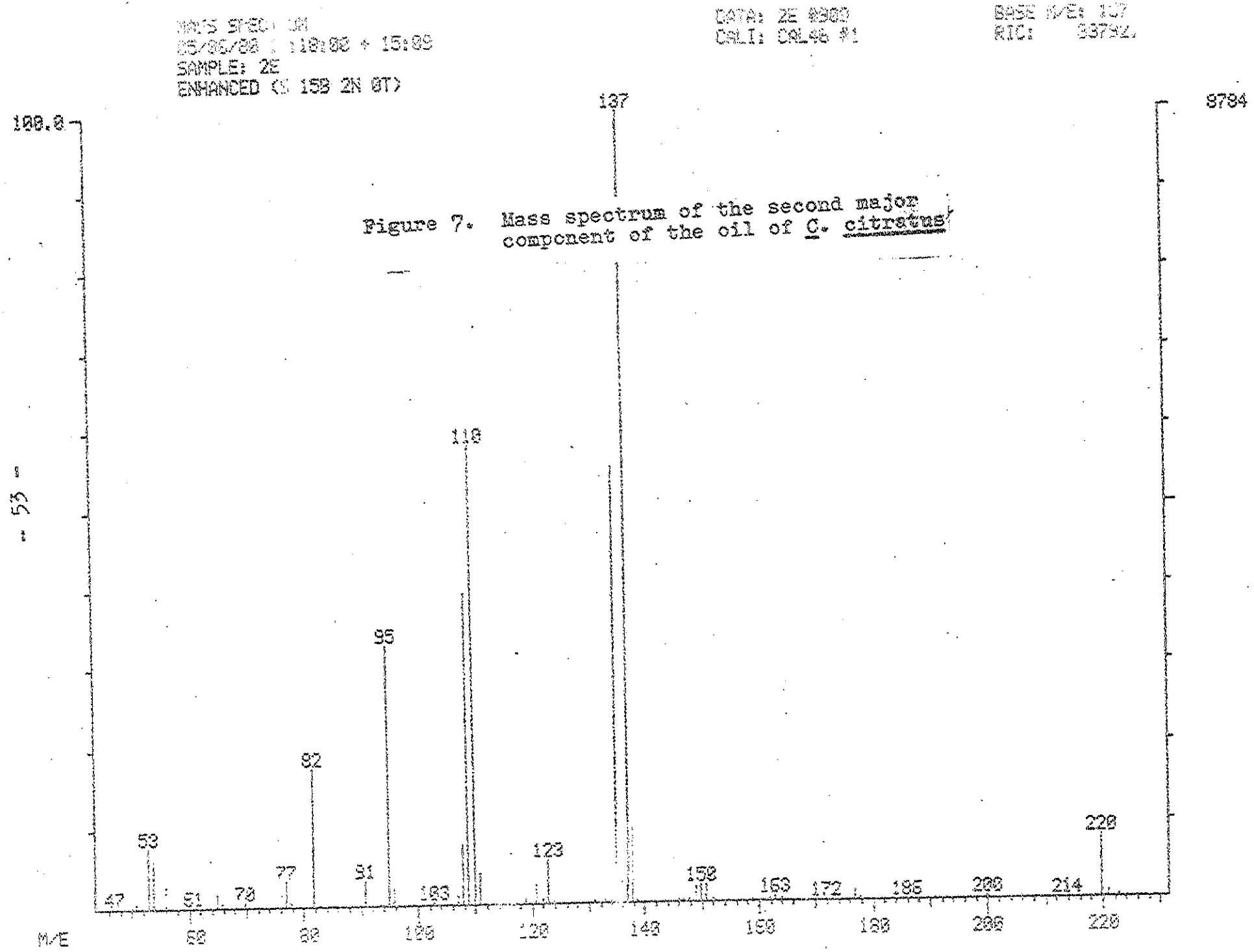

Figure 7. Mass spectrum of the second major component of the oil of *C. citratus*

7. Experimental

Isolation of the oils from the plants were carried out on a modified Stark and Dean apparatus. Optical rotations were determined on Ivac polarimeter. Refractive indices were determined on Atago refractometer. Specific gravity of the oils were determined using a pyconometer. Infra-red spectra were determined as thin films using a Perkin-Elmer model 727B grating spectrophotometer. Ultraviolet - visible spectra were taken on Pye Unicam model SP8-100 spectrophotometer. Nuclear magnetic resonance spectra were obtained in carbontetrachloride (CCl_4) on a Varion T-60A spectrometer and are reported as ppm values relative to tetramethyl silane (TMS) internal standard. Mass spectra were determined on a Finnigan model 3200F GC/MS spectrometer attached with Finnigan model 6100 data system. The eight most intense peaks are reported. Gas chromatograms were obtained on a Hewlett Packard model 5710A gas chromatograph equipped with flame ionization detector (FID).

Analytical thin layer chromatography (tlc) were run on a 0.25 mm thick layer of silica gel GF_{254}(Merck). Components were detected by their ultraviolet fluorescence. Column chromatography were performed using silica gel 60(70-230 mesh) Merck .

Standard mixture was prepared from standard samples of 0.05 ml each of α-pinene, camphene, β-pinene, myrcene, 1,8 cineole, γ-terpinene, fenchone, menthone and geraniol and 25 mg of each of borneol, menthol and camphor. These samples were obtained from Dresden University of Technology, Department of Chemistry, GDR.

7.1 *Artemisia rehan* Chiov

Family: Asteraceae

Local name: 'Ar'ti'

A specimen of this plant is available in the Herbarium of Addis Ababa University with a collection number Berhanu-1.

7.1.1 Isolation of the oil

A. rehan obtained in December 1979 from 'Marcato' and 'Sabata' around Addis Ababa (911 gm) was cut to suitable size to put into 5 litres round bottom flask fitted with a modified Stark and Dean distilling apparatus. This was steamdistilled for 4 hours. This gave 1.80 gm oil (0.20%) (density 0.9123).

7.1.2 Fractionation of the Oil into a Hydrocarbon and Oxygenated Compounds.

The oil (4 gm) was run on 120 gm column of silica gel. This was eluted successively with 600 ml of distilled n-hexane and ethylacetate. Fractions, 50 ml, were collected. The solvents were removed by distillation on a steam bath. Each fraction was then subjected to GLC analyses.

7.1.3 GLC analyses

Stainless steel, and copper columns 6' x 1/8'' outer diameter (o.d) packed with

10% Carbowax 20M on chromosorb WHP 60/80 mesh and 10% Ucon 50 HB 2000 on chromosorb WHP 80/100 mesh respectively were used in these analyses.

The standard samples and the standard mixture were chromatographed on both columns at isothermal and programmed oven temperature. Similarly, the oil and the fractions obtained were chromatographed under the following conditions.

Column temperature:

a. Isothermal - 180°C

b. Programmed

Intial temperature 70°C
Final temperature 200°C, isothermal 4 min.
Rate: 2°C/min,

Inlet temperature: 200°C

Detector temperature: 250°C

Chart speed: 15 inches/hour

Carrier gas (N_2): 30 ml/minute

H_2: 30 ml/min.

Air: 240 ml/min.

Injection volume: 1 µl

7.1.4 Isolation of Chamazulene

The oil (4 gm) was run on 120 gm column of silica gel. This was eluted successively with 600 ml of distilled n-hexane and ethylacetate.

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Fractions of 50 ml were collected. The blue component was clearly observed on the column as it separated leaving the yellow mixture of the oil. The blue component appeared in fractions seven, eight, nine and ten of hexane eluents. Each fraction was run on tlc and all gave a single spot. (Solvent system: n-hexane: ethylacetate: 26: 1, Rf: 0.59). This component gave a single peak on Ucon and did not appear on Carbowax.

Nmr spectrum: 7.88 ppm (d, 1H), 6.77 ppm (d, 1H) 7.17 ppm (dd, 1H), 7.33 ppm (d, 1H), 6.9 ppm (d, 1H), 2.55 ppm (s, 3H), 2.7 ppm (s, 3H), 1.3 ppm (t, 3H).

UV-Visible spectrum: broad absorption at 590-660 nm and sharp peaks at 366, 350, 304, 284 (max), 244 nm.
Mass spectrum: (M^+/e 184).
Mass: 169, 184, 153, 155, 128, 152, 77, 115
Relative Intensity: 100, 66, 44, 38, 34, 34, 32, 31
ir: 2975cm^{-1}, 3100 cm^{-1}

7.1.5 Determination of Chamazulene in the Oil of A. rehan

Solutions of the isolated chamazulene in 95% ethanol, 0.60 x 10^{-5}M and 0.30 x 10^{-5}M were prepared. The absorbance of each of the above solutions 1.018 and 0.520 respectively were obtained at λ max (600 nm). The extinction coefficient was calculated to be 187.1. The absorbance of 0.5 ml of the oil in 10 ml 95% ethanol was found to be 1.078.

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The extinction coefficient and this absorbance reading were then used to calculate the concentration of chamazulene in the oil of A. rehan. (0.26%)

7.1.6 Isolation of Davanone

Fractions two and three obtained with ethylacetate elution during column chromatography of A. rehan oil which exhibited the same components on GLC were combined (550 mg). The combined mixture was chromatographed on 30 gm of silica gel. This was successively eluted with ethylacetate: n-hexane:: 1:20, 1:10, 1:5, 1:1 (200 ml each). 50 ml fractions were collected. The 12th fraction gave a major component (ca. 80%) and the other fractions exhibited several components on GLC. The major component isolated was further analysed using ir, nmr and ms and was identified as davanone.

ir: 1710, 1640, 915, 990 cm^{-1}

nmr: 0.88 ppm (d, 3H), 1.17 ppm (s, 3H), 1.56 (s, 3H) 1.66 ppm (s, 3H), 2.5 ppm (m, 1H), 4.73 (d, 1H), 4.92 (d, 1H), 5.77 (dd, 1H).

ms: (M^+/e: 236)

mass: 111, 93, 55, 69, 94, 83, 79, 57

Relative Intensity: 100, 50 27, 24, 18, 5, 4, 3.

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7.1.7 GLC/MS analyses

The oil of A. rehan and the fraction obtained from column chrometography were subjected to GLC/MS on a glass column packed with 10% carbowax 20M chromosorb WHP 100/120 mesh and on capillary column coated with carbowax 20M. GLC/MS conditions and the eight most intense peaks are given below.

a. GLC condition

Initial temperature 100°C

Final temperature 200°C

Rate 8°C/min.

Carrier gas (He): 30 ml/min.

b. MS conditions

Relative Intensity 10

Sensitivity 10^{-6} amps/sec.

First mass 1 amu

Last mass 300 amu

Integral start 40 amu

Electron energy 70eV.

1. Bornyl acetate (M^+/e: 196)
   Mass: 95, 93, 121, 136, 55, 80, 69, 108
   Relative Intensity: 100, 45, 32, 28, 19, 18, 15, 14

2. Camphor (M^+/e: 152)
   Mass: 111, 93, 55, 69, 94, 83, 79, 57
   Relative Intensity: 100, 50, 27, 24, 18, 5, 4, 3

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13. (M^+/e 234) - not identified
Mass: 43, 104, 91, 109, 105, 45, 107, 55
Relative Intensity: 100, 75, 72, 55, 44, 44, 42, 42

7.2 Cymbopogon citratus (DC) Stapf.

Family Poaceae (Graminae)
Synonyms: Andropogon citratus, Andropogon schoenanthus
Common name: Lemon-grass
Local name: 'Tej Sar'

This is a tufted perrenial upto over 200 cm high with rigid leaf blades upto 100 cm. long, fulliform or narrowly linear.[78] The specimen of this plant is available in the Herbarium of Addis Ababa University with a collection number Paulos-1.

7.2.1 Isolation of the Oil

Cymbopogon citratus collected in July 1979 (176 gm) from Addis Ababa was distilled in the same manner as described for Artemisia rehan to give 7.60 gm of the oil (4.32%).

7.2.2 Fractionation of the Oil into Hydrocarbon and Oxygenated Compounds

The oil (1 gm) was chromatographed on 60 gm column of silica gel in the same manner as specified for A. rehan.

GLC and GLC/MS analyses were again carried out in the same manner as described for oil of A. rehan and eight most intense peaks are recorded below for the compounds identified in this oil.

1. Δ^3-Carene or Ocimene (M^+/e 136)
   Mass: 93, 91, 79, 92, 80, 53, 105
   Relative Intensity: 100, 47, 46, 41, 40, 18, 18, 15

2. Citral a (M^+/e 152)
   Mass: 69, 84, 94, 83, 53, 67, 109, 70
   Relative Intensity: 100, 26, 13, 9, 9, 7, 6, 6.

3. Citral b (M^+/e 152)
   Mass: 69, 94, 84, 109, 67, 59, 95, 82
   Relative Intensity: 100, 28, 26, 20, 20, 20, 19, 18

4. Citronellol (M^+/e 156)
   Mass: 69, 55, 67, 81, 82, 95, 56, 71
   Relative Intensity: 100, 62, 55, 52, 37, 34, 32

5. Geraniol (M^+/e 154)
   Mass: 69, 68, 93, 67, 80, 121, 136, 53
   Relative Intensity: 100, 41, 21, 13, 11, 10, 9, 6

6. Linalool (M^+/e 154)
   Mass: 71, 93, 69, 80, 67, 83, 121
   Relative Intensity: 100, 61, 56, 41, 28, 17, 16, 13

7. Nerol (M^+/e 154)
   Mass: 69, 68, 93, 67, 121, 80, 53, 85
   Relative Intensity: 100, 41, 21, 12, 9, 9, 6, 6

8. Terpinolene (M^+/e 136)
   Mass: 71, 93, 55, 69, 79, 67, 53, 92

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Relative Intensity: 100, 63, 55, 40, 30, 20, 15, 14

9. (M^+/e 154) - not identified
   Mass: 43, 55, 81, 69, 71, 57, 41, 83
   Relative Intensity: 100, 59, 59, 55, 48, 41 39

10. (M^+/e 170) - not identified
    Mass: 71, 43, 41, 55, 93, 69, 57, 80
    Relative Intensity: 100, 87, 67, 60, 53, 43, 37, 0.8

11. (M^+/e 220) - not identified
    Mass: 137, 110, 135, 99, 95, 82, 138, 220
    Relative Intensity: 100, 58, 56, 39, 33, 18, 9, 8

12. (M^+/e 222) - not identified
    Mass: 41, 69, 45, 43, 73, 57, 68, 55
    Relative Intensity: 100, 98, 81, 62, 47, 45, 42, 40

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