Analysis of the Essential Oils of

Tagetes minuta L.

and

Artemisia rehan Chiov.

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ABSTRACT

Analysis of the Essential Oils of
Tagetes minuta
and
Artemisia rehan

By
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Research Advisor: Dr Ermias Dagne

The chemical compositions of the essential oils of the leaves and flowers of *Tagetes minuta L.* were determined by chromatographic and spectroscopic methods. The characteristic components of the essential oils produced from the leaves of *T. minuta* were found to contain (Z)-Ocimenone (41%) and (E)-Ocimenone (18%) as the major components, while the oils from the flowers of *T. minuta* contained *cis*-β-ocimene (46%), (Z)-and (E)-Ocimenones (22% and 10%, respectively).

The volatile oil constituents of the aerial parts of *Artemisia rehan* plants purchased at different times were investigated using gas chromatographic and spectroscopic techniques. Differences in the camphor (5%, 30%, 31%), chamazulene (7.6%, 1.9%, 1%) composition and yield (0.3%, 0.4%, 0.8%) of these oils was observed. Such differences in the essential oil composition and yield may be ascribed to the existence of different *A. rehan* chemotypes, seasonal variation or difference in stages of development of the plant material.

1. INTRODUCTION

It is a common experience that flowers, fruits, leaves, stems and roots of many plants have some pleasant smell. Steam distillation or solvent extraction of these plants or their parts give sweet-smelling oils, called essential oils. Essential oils or ethereal oils, which are the volatile components of plants are mostly obtained by steam distillation. In some cases these oils may also be isolated by other techniques such as extraction, expression, maceration, etc.

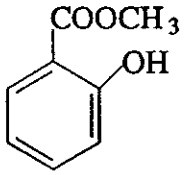
The function and precursors of the essential oils in plants are still not satisfactorily explained. Whether they serve as attractants, repellents or protectants or are simply waste products is obscure [1,2].

The term "*essential oil*" is derived from the consideration that the oil has the same characteristic "*essence*" as the plant it is derived from [3]. These essential oils are to be distinguished from the "fixed oils" such as those used for domestic cooking or the "mineral oils" used as raw materials in the petroleum industry. Essential oils consist of terpenoids and aromatic hydrocarbons and their oxygenated derivatives and are necessarily of plant origin. A few contain nitrogen and sulphur. Example: oil of mustard contains organic isothiocyanate, garlic and onion oils contain organic sulphides [1,4]. The relatively rapid evaporation and pronounced odour, aside from their chemical composition, differentiate fundamentally essential oils from the fixed, fatty oils. But fixed oils may be of plant origin and are essentially triglycerides, i.e. triesters of glycerol while the mineral oils are predominantly complex hydrocarbons derived from the remains of tiny sea animals laid down in past geologic ages [3,4].

The essential oils are closely associated with the history of man's civilization. They were utilized in the ancient civilization of Arabia and Asia as perfumes, deodorants and in the preservation of the remains of the dead [5].

Essential oils have varied industrial and pharmaceutical applications. They are used to give odour or flavour to a wide range of products including cosmetics and perfumes, foods, detergents, pharmaceutical, lacquers and dyes and also mask the natural odours of materials such as plastics, rubber goods, artificial leathers and paints [6,7].

Great progress has been made in the synthesis of individual components of essential oils in the chemical laboratories and these syntheses have been blended together in an attempt to duplicate the oils themselves. But such reconstituted oils usually lack certain of the odour notes of the natural products because of the absence of trace ingredients, often unidentified, that may be present in the natural oils, and also tend to have more "chemical odour" due to trace impurities in the syntheses that are different from the components of the natural oils of plant origin. Example: synthetic methyl salicylate (1) can be differentiated by odour and taste from oil of sweet birch even though the natural oil contains 98% methyl salicylate [8].



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Ethiopia, because of its clearly distinct agro-climatic regions favourable to the growth of different essential oil bearing plants, has got a variety of odoriferous plants which are used as medications, spices and condiments [9]. But today Ethiopia occupies a very insignificant position in the international essential oil trade. Thus efforts must be made to identify these aromatic plants that yield essential oils like those that are available in the international market. Since the yield, aroma, quality and flavour of these natural oils are affected by different environmental conditions [10,11,12], the composition and major components of these volatile oils must be

identified which of course determines their market value. Therefore, the analysis and characterization of the chemical constituents of Ethiopian aromatic plants will help to asses the quality of the essential oils and to generate scientific and technical data for the development of commercial scale production of essential oils in the country.

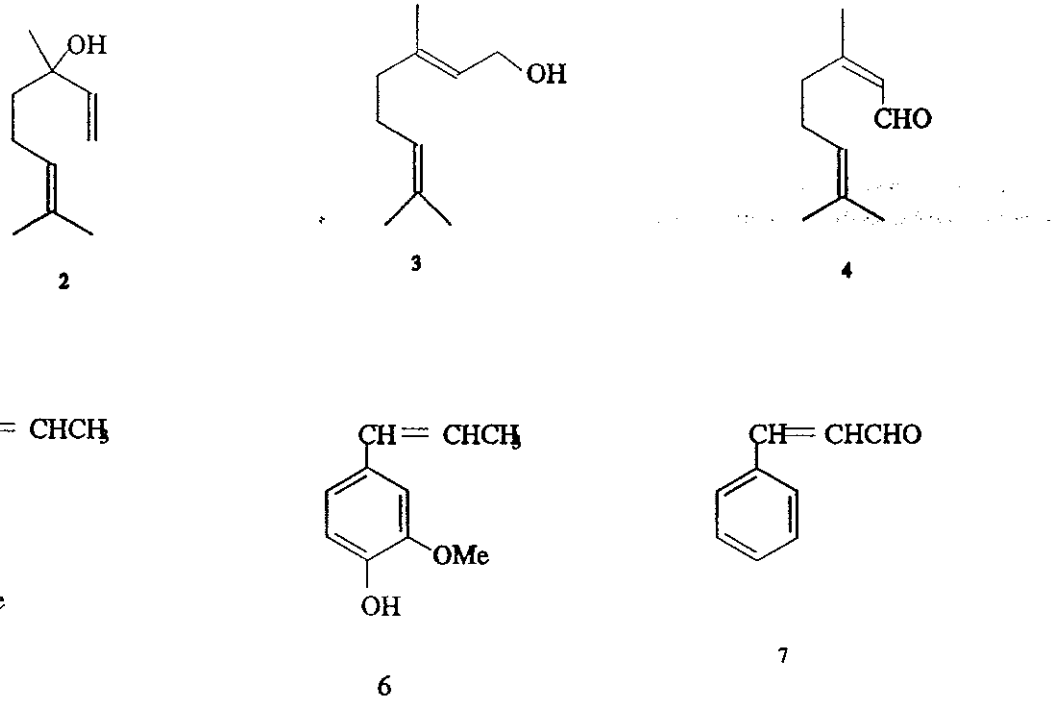
This study deals with two plants: *Tagetes minuta L. and Artemisia rehan Chiov.,* and it is the first study where the essential oil components of wild *Tagetes minuta* of Ethiopian origin have been analyzed.

The plants *Tagetes* and *Artemisia* were known to produce essential oils since a long time. Distillation, comparison of yield and determination of their physico-chemical properties began to be made in the early 1900s [13,14,15]. But the advent and development of chromatographic procedures especially Gas-Liquid chromatography (GLC) and Thin Layer Chromatography (TLC) as routine analytical techniques during the 1960s has facilitated the precise identification and determination of these essential oils. That is why people in this area are still re-examining the volatile oil constituents of higher plants.

1.1 Composition of Essential Oils

The components of essential oils belong to many classes of organic compounds, particularly the terpenoids. The common feature of the terpenoids is that the carbon skeletons of their molecules are built up of isoprene (C_5) units. Hence they are classified into different categories depending on the number of isoprene units in the molecule, a single "terpenoid unit" being equal to two isoprene units [16]. Of the terpenoids, the mono- and sesquiterpenoids, which are the C_{10} and C_{15} representatives of the terpenoid group, are the quantitatively more important components of essential oils from higher plants [17]. The biosynthesis of the C_{10} and C_{15} representatives of terpenoids is discussed elsewhere briefly [18-22].

In some essential oils, a terpenoid is the major component e.g. rose oil contains about 85% linalool (**2**), palmarosa about 84.9% geraniol (**3**) and lemongrass oil over 75% citral(**4**). Other essential oils contain large amounts of non terpenoid components. Methyl salicylate makes up about 98% of oil of sweet birch, anethole (**5**) about 90% of anise oil, and eugenol (**6**) as much as 95% of clove oil [4]. Most of the essential oils are mixtures, some of 50 or more constituent compounds. However, a few consist of virtually a single compound e.g. oil of sweet birch consists of methyl salicylate and Cassia [6] of cinnamaldehyde (**7**).



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5 6 7

Figure 1. Major terpenoid components of some essential oils.

1.2 Isolation of Essential Oils

The following four methods are employed for the isolation of essential oils which are subsequently separated into individual components employing physical or chemical methods.

A. Expression Method

The plant material is crushed and filtered to remove the solid particles. The filtrate is subjected to high speed centrifuge and the clear essential oil is obtained at the top.

B. Steam Distillation

Essential oils are most often separated from different parts of plants by steam distillation at atmospheric pressure. The oils are insoluble in water and can easily be separated . Most of the flavour and aromatic compounds are found in the oil layer, little being present in the water. But this method has got its own disadvantage because some essential oil constituents are thermo-labile, and structural changes can take place during isolation and hence converted to non- or less fragrant products during the course of steam distillation. Fortunately, most of the essential oils of commerce are steam volatile, reasonably stable to action of heat and practically insoluble in water and hence suitable for processing by steam distillation.

C. Solvent Extraction

The essential oils which are sensitive to heat are extracted from the plant material with low boiling solvents such as petrol at room temperature. Distillation of the solvent from the filtered extract under reduced pressure leaves the essential oil. Extracted flower oils truly represent the natural perfume oils as originally present in the flowers. All extracted flower oils are of more or less dark colour because they contain much of the natural plant pigments which are not volatile.

D. Adsorption Method

The petals of flowers are left in contact with a fat preparation (a mixture of lard and tallow) for several days at about 50°C, until the fat is saturated with the essential oil. The saturated fat is then extracted with EtOH and the solvent

removed under reduced pressure at 0°C. Recently activated charcoal has been used instead of fat [23].

After isolation of the essential oils, which are mixtures of a number of compounds, the constituents are separated by a variety of methods depending up on their physical or chemical properties.

1.3 Chemistry and Characterization of Essential Oils

Since in the early stages of research it was difficult to define sharply the isolated compound, a great number of terpenoids were named after the plant from which they were obtained. Characteristic for many of the essential oil constituents is their instability and the ease with which intra-molecular rearrangements occur. These properties have been a great hindrance to the study of these compounds. Thus the analysis of essential oils is representative of types of complex problems which can be solved using different analytical methods, either individually or in combination.

More recently, chromatography (in its various forms) has been used both for isolation and separation of essential oils. Gas-Liquid Chromatography (GLC) has been particularly useful in the identification of the constituents of essential oils but prior to GLC analysis the hydrocarbons and oxygenated components of the oil can be separated using column chromatography. Hydrocarbons are eluted with hexane or petrol and on successive elution with EtOAc the oxygenated derivatives are obtained.

Another method called the slurry method is employed to separate the hydrocarbons from the oxygenated compounds. Silicic acid is added to the essential oil dissolved 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 EtOAc [9].

The fractions obtained via the above methods are then subjected to GLC analysis. In GLC analysis, the interval of time between the introduction of the mixture onto the column and the elution of a component, usually called retention time, is characteristic for a compound which depend upon its solubility in the stationary phase [24]. It would therefore be reasonable to infer that one could identify the essential oil components by direct comparison of their retention times with standard samples or published data. As the retention time is subject to variation and its value is dependent upon column temperature, the carrier gas flow rate (both of which may alter between the calibration and analytical measurements), column length and the type and loading of the stationary phase [24], any direct comparison with standard samples and the literature data could be erroneous. The observed peak could be that of another compound which by coincidence has the same retention time and together giving the appearance of a single peak.

Unequivocal identification of an unknown component is possible by co-injection chromatography (peak enhancement method) coupled with the retention data obtained from two or more different stationary phases. If, however, the added compound gives an extra peak with a retention time different from those of the components of the mixture then one has a conclusive evidence for the absence of that compound in the mixture.

A still better method of analysing essential oils is by using a gas chromatograph interfaced with a mass spectrometer (GC-MS). The impact of GC-MS has been tremendous, adding the power of mass spectrometry in structure elucidation to the power of GC to deal with small size and multicomponent mixtures of complex compounds. Here compounds are identified on the basis of molecular weights, fragmentation pattern and retention times. Since odour will always remain an important criterion [1], no assay of an essential oil will be complete without careful organoleptic tests which, however, require considerable experience.

1.4 Essential Oils of the Genus *Tagetes*

The genus *Tagetes* (marigold) with nearly 40 species embraces a diversity of plants with yellowish green leaves, greenish yellow flowers and strong distinctive smell belonging to the family *Compositae* [25,26]. The natural range for all of the *Tagetes* species is from South Western United States to Argentina, although it should be noted that *T. minuta* has become widespread throughout the world [26]. These plants contain a reddish yellow liquid oil with a powerful, peculiar and disagreeable odour which can be isolated by steam distillation. The essential oils of *Tagetes* species have been successfully used in oriental confectioneries, in the soap and cosmetic industry, and as a modifier in hair lotions [27,28]. The *T. minuta* oil displayed potent antibacterial activity against both gram-negative and gram-positive bacteria [29-32].

1.4.1. *Tagetes minuta L.*

Tagetes minuta L. (Syn. *T. bonariensis pers.; T. glandulifera Schran.; T. glandulosa Schrank ex Link.; T. porchyllum Vell.; T. riojana Ferraro*), found in the sub genus *Tagetes*, is an annual aromatic plant which grows in the summer, attains a height of *ca.* 150 cm under favourable conditions and tends to lose its leaves and become very "stalky" after producing small, spindle-shaped seeds which shatter very easily [26,33]. The plant was introduced from South Africa to East Africa during the East African campaign (1914 to 1918) where it took root and is still spreading over wide areas - a real nuisance to planters [27]. *T. minuta* which is locally known as "*Gimie" or "Ye-ahiya Choma*" thrives along road sides and on any land that has been ploughed up. In Africa it is claimed that the plant acts as a fly and vermin repellent . The natives of East Africa hang Tagetes plants in their huts to keep out the swarms of flies [27].

A survey of the chemical literature reveals that in 1924 a sample of South African *Tagetes* oil was thought to contain carvone (**8**), linalool (**2**), linalyl acetate (**9**), myrcene (**10**)

and ocimene (**11**) [26]. A year later Jones and Smith used fractional distillation and derivatization to determine that the oil of the flowering tops of *T. minuta* contained limonene **12** (3%), ocimene (**11**) (3%) and tagetone (**13**) (50-60%) [26]. The following year, Jones intimated that two isomers of tagetone were probably present in *Tagetes* oil and same person in 1934 further determined that a second ketone, dihydrotagetone (**14**) (5-10%) was also found as a constituent of *Tagetes* oil [26]. In 1951, Fester *et al.* confirmed the earlier findings of Jones and Smith by showing that an oil of *T. minuta* grown in Argentina contained ocimene (**11**), tagetone (**13**), and dihydrotagetone **14** [26]. Twelve years later, Handa *et al.* [34] used column chromatography and chemical derivatization to tentatively identify aromadendrene (**15**), tagetone (**13**), phenylethyl alcohol (**16**), ocimene (**11**), salicylaldehyde (**17**), phenyl acetaldehyde (**18**), 2-hexenal (**19**), eudesmol (**20**), linalyl acetate (**9**) and trace amounts of limonene **12** and linalool **2** in an oil of *T. minuta* produced from plants found growing wild in the North Western region of India.

In 1970, Demucciarelli and Montes [35] found that an Argentinian oil of *T. minuta* contained tagetone (**13**), limonene (**12**), ocimene (**11**), linalool (**2**), carvone (**8**), β-pinene (**21**), α-pinene (**22**), camphene (**23**), α-phellandrene (**24**), acetic acid, formic acid, acetates, formates, aromatic acids, geraniol (**3**), phenyl ethanol (**16**) and salicylaldehyde (**17**). In the same year, Kekelidze [36] examined an oil of *T. minuta* of Russian origin and found that it contained α-pinene (**22**), sabinene (**25**), myrcene (**10**), limonene (**12**), ocimene **11** (50%), *p*-cymene (**26**), geraniol (**3**), neral (**4**), linalool (**2**) and α-terpineol (**27**).

In 1973, Carro de la Torre *et al.* [37] reported that the oil of T. minuta produced in Argentina was found to contain α-pinene (**22**), β-pinene (**21**), limonene (**12**), linalool (**2**), citral (**4**), phenylethyl alcohol (**16**), salicylaldehyde (**17**), tagetone (**13**), ocimene (**11**), formic acid, acetic acid, valeric acid (**28**) and camphene (**23**). Two years later, Gupta and Bhandari used TLC

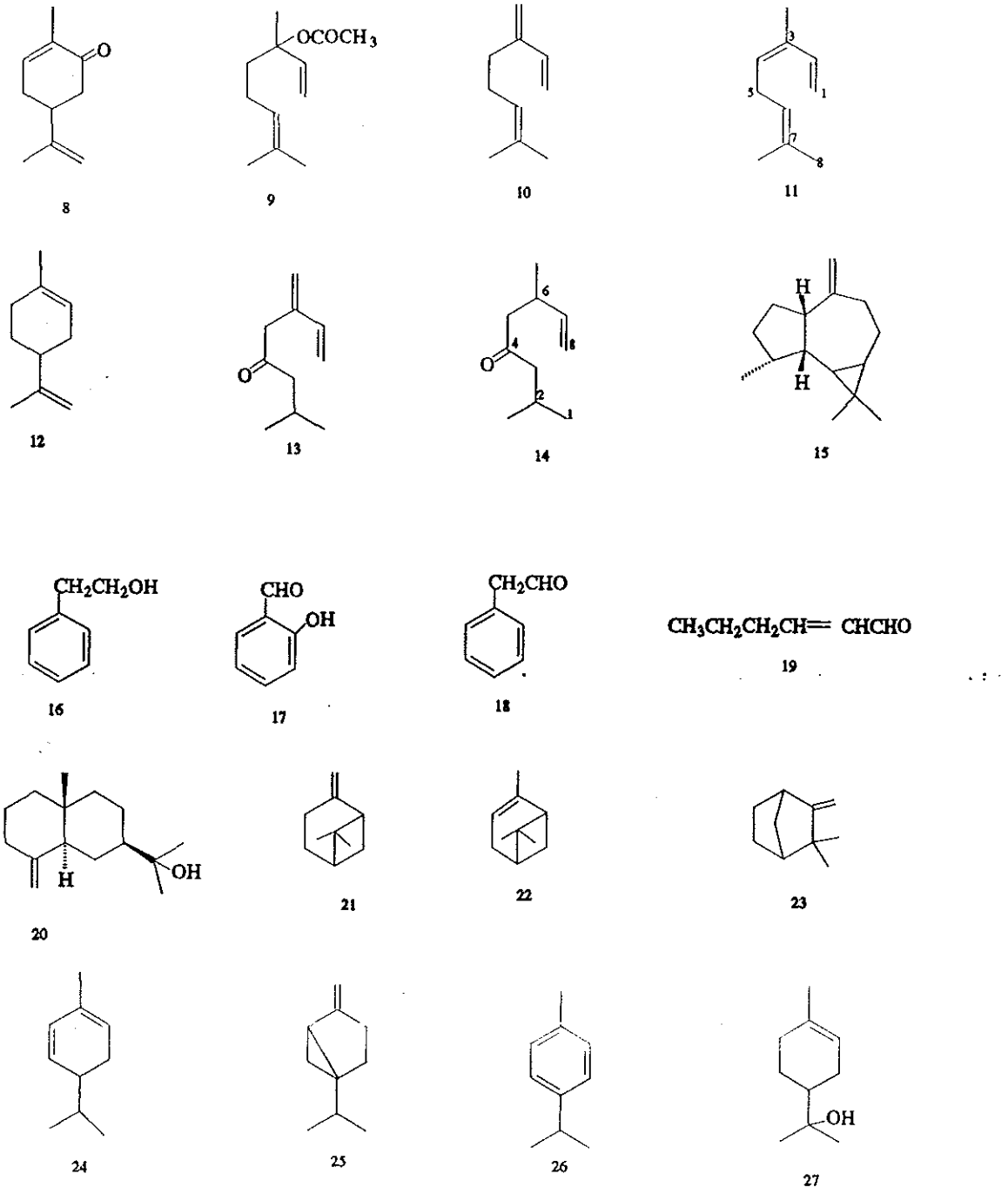


Figure 2. Some essential oil components reported to occur in *T. minuta* oil.

and derivatization to determine that the oil of *T. minuta* produced in India contained limonene (**12**), ocimene (**11**), myrcene (**10**), aromadendrene (**15**), linalool (**2**), linalyl acetate (**9**), carvone (**8**), 1,8-cineole (**29**) and salicylaldehyde (**17**)

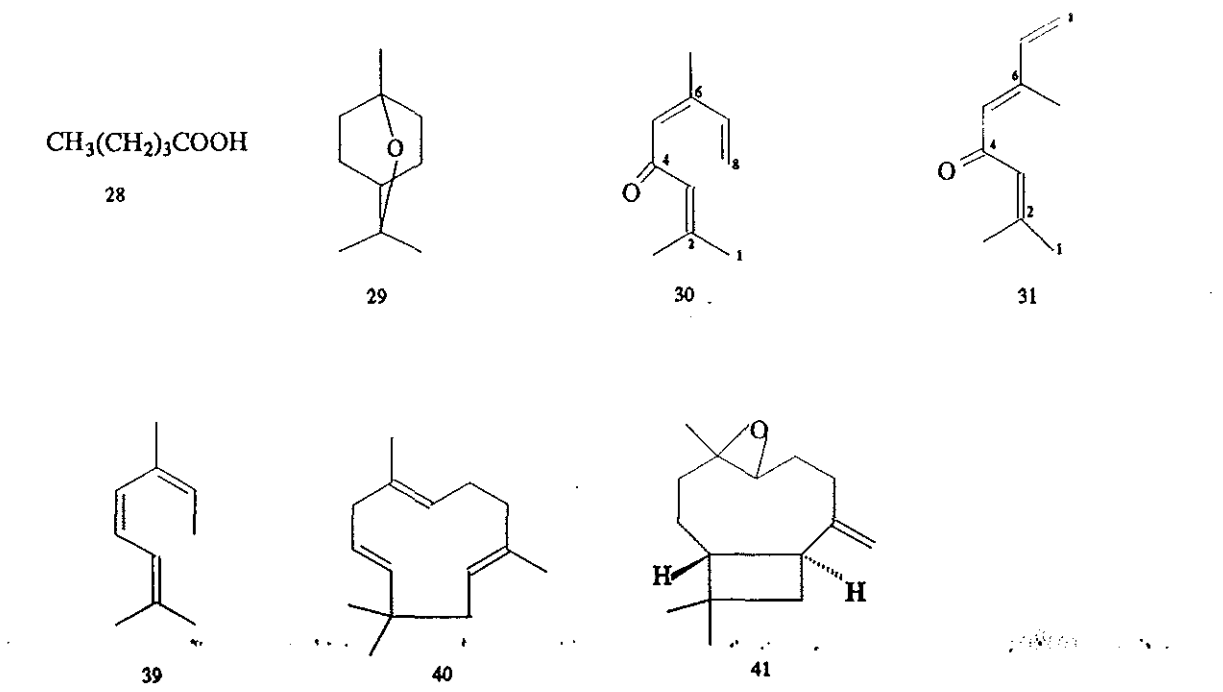


Figure 2. Cont'd.

[26]. In 1976, Koketsu *et al.* [38] reported that commercial oil of Brazilian *T. minuta* contained more ocimene and much less dihydrotagetone than the non commercial oil and also showed that the commercial sample revealed a large quantity of unsaturated hydrocarbons. Five years later, Baslas and Singh [39] reported that an Indian oil of *T. minuta* was found to contain aromadendrene (**15**), ocimene (**11**), myrcene (**10**), and tagetone (**13**) as the major constituents. The following year Siqueira *et al.* [40] showed that *T. minuta* oil produced in Brazil was found to contain tagetone (**13**), limonene (**12**), *p*-cymene (**26**), and α-pinene (**22**).

In 1983, Downum and Towers reported that an extract of *T. minuta* was found to contain four of the thiophenes common to the *Tagetes* genus [26]. A year later, Maurer and Hauser [41] used modern spectroscopic techniques to characterize a new

aldehyde 5-isobutyl-3-methyl-2-furancarbaldehyde for the first time in *T. minuta* oil. In 1986, Hethelyi *et al.* [32] reported that *T. minuta* oil contained β-ocimene (**11**), dihydrotagetone (**14**), tagetone (**13**), and (Z)-ocimenone (**30**) and (E)-ocimenone (**31**). In the same year, Lawrence *et al.* [42] showed that North American *T. minuta* oil was found to contain *cis*-β-ocimene (**11**), dihydrotagetone (**14**) and Hethelyi *et al.* [31] revealed β-ocimene (**11**), dihydrotagetone (**14**), tagetone (**13**) and (E)-ocimenone (**31**) as the major components. In 1987, Hethelyi *et al.* [43] also confirmed that oil of *T. minuta* contained β-ocimene (**11**), dihydrotagetone (**14**), tagetone (**13**) and (Z)-ocimenone (**30**) and (E)-ocimenone (*31*). That same year, Hethelyi *et al.* [10] conducted comparative analysis of the essential oils of various origin and indicated that the Tagetes species contain an oil with different but specific composition. Only quantitative and not qualitative variations were found. In 1992 Singh *et al.* [44] reported that the flowers of *T. minuta* were found to contain (Z)-β-ocimene (**11**), dihydrotagetone (**14**), (Z)-tagetone (**13**), (E)-ocimenone (**31**) and (Z)-ocimenone (**30**).

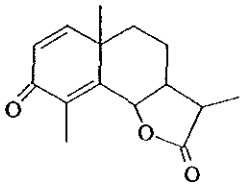
From a pharmacological stand point, it was found in 1941 that essential oils of *T. minuta* caused transitory symptoms of listlessness and anorexia in sheep [26]. A few years later it was shown that *T. minuta* was found not to be a repellent to the Austrian sheep blowfly even though it had been implicated [26]. In 1951, Australian wild *T. minuta* oils were screened to see if they would enhance Pyrethrum toxicity and it was found out that the leaf oil of *T. minuta* caused a large loss of Pyrethrum activity [26]. In 1969, Chandhoke and Ghatok [45] showed that the oil of *T. minuta* exhibited bronchodilatory, tranquillizing, hypotensive, spasmolytic, and anti-inflammatory properties. In 1973, it was shown that an oil of *T. minuta* possessed juvenile hormone activity on *Dysdercus koenigii* and five years later it was observed that 40 ppm concentration of a component of *T. minuta* oil, (5E)-ocimenone, caused 100% mortality of mosquito (*Aedes aegypti*) larvae [46]. In 1983, Maynadier *et al.* reported that two thiophenes 5-(3-buten-1-ynyl)-2,2′ bithienyl and α-terthienyl from *Tagetes* oil were shown to

a search for new sources of santonin (**32**), with the result that this compound is known as a constituent of about 20 species of *Artemisia*. Recent years have seen an expansion of studies of *Artemisia* as a part of an increasing interest in the chemistry of plants of the *Compositae*, principally with respect to the sesquiterpenoid lactones, a class of compounds almost unique to this family [49]. The *Artemisia* plants are strongly scented herbs with pale yellow flowers [25] and contain a blue [9,51] or light yellow liquid oil with a powerful scent obtained by steam distillation. These volatile oils are very important flavouring ingredients in the formulation of alcoholic liquors of the absinthe type and in vermouth [54,55].

1.5.1 *Artemisia rehan Chiov.*

Artemisia rehan locally known as "*Ariti*" is a perennial odorous herb which is widespread in and native to Ethiopia [9]. It is used to be the custom for Ethiopian women to carry to church large bunches of this plant, because the aromatic scent helps prevent feeling of drowsiness. This plant contains a deep blue liquid oil with a pleasant smell that can be isolated by steam distillation. The blue coloration of the oil is due to the presence of chamazulene (**33**).

Survey of the chemical literature indicates that in 1923, Paolo [56] showed cultivated *A. rehan* from Ethiopia yielded a volatile oil with an odour similar to that of the Indian Davana oil containing cinnamyl alcohol (**34**), cinnamic acid (**35**) and cadinene (**36**). In 1982 Abegaz and Paulos [9] identified 22 components in the oil of *A. rehan* using GC, UV and ^{1}H NMR spectroscopic techniques with davanone (44.36%) (**37**) and camphor (23.49%) (**38**) as the major components.



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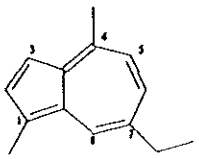

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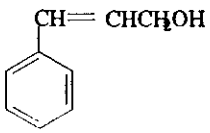

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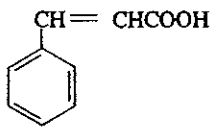

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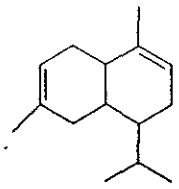

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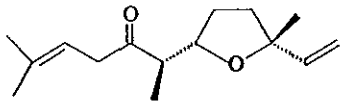

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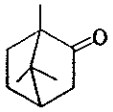

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So far the following components are reported in the literature as constituents of the essential oils of *A. rehan* [9].

Bornyl acetate, camphene, camphor, chamazulene, 1,8-cineole, davanone, *cis*-ethyl cinnamate, *trans*-ethyl cinnamate, eudalene, eudesmol, linalool, limonene, menthol, menthone, myrcene, α-pinene, β-pinene, γ-terpinene, terpinen-4-ol, α-terpineol.

1.6 Objective of the Project

The objective of this work is to analyze and characterize some of the chemical constituents of the essential oils of *Tagetes minuta* and *Artemisia rehan* responsible for their aromas and flavourings. The generated chemical data in the course of this work may help in the assessment of the quality of the essential oils derived from *Tagetes minuta* and hence the locally available wild aromatic plant (*T. minuta*) may be cultivated for future commercial scale production of essential oils.

2. RESULTS AND DISCUSSION

The analysis of the oils obtained from the leaves and flowers of *T. minuta* and aerial parts of *A. rehan* was mainly carried out with Gas Liquid Chromatography using programmed column oven temperatures and NMR spectroscopy. Identification of some of the peaks and components was done mainly by comparison of the retention times with standard samples and further verified by peak enhancement techniques.

2.1 Tagetes minuta L.

T. minuta plants were collected in September, 1995 from Kottebe during their flowering stage. The leaves and flowers were separated and subjected to steam distillation. The oils isolated from the leaves and flowers were coded as TM-L and TM-F, respectively. The physico-chemical properties of TM-L and TM-F are shown in Table 1.

The oils from the leaves and flowers of *T. minuta* manifest similar and characteristic disagreeable odour. The oils turn thick on exposure to air, the degree of thickening being greater in the leaf oils. The analysis of these oils was carried out mainly using ^{13}C NMR spectroscopy and GC. Under programmed oven temperatures, the chromatograms of the leaf and flower oils of *T. minuta* revealed 23 and 30 components, respectively (Figure 3).

Table 1: Physico-chemical properties of TM-L and TM-F

Oil	Colour	Yield (%)	d	n	$[\alpha]_D$
TM-L	Yellowish red	0.6-0.9	0.9	1.54	+2.1
TM-F	Slightly yellowish red	0.6-0.8	0.8	1.51	+1.0

Legend: d: Specific gravity at 20°C
n: Refractive index at 20°C
[α]: Specific rotation at 25°c

Comparison of retention times with reference samples, co-injection chromatographic experiments and spectroscopic analyses on these oils resulted in the identification of the compounds listed in Tables 2 and 3.

Table 2: Identified compounds with their retention times and percentage composition from the oil of TM-L.

Substance	R_t (min)	Percent Composition	Method of Identification
1. 1,8-Cineole (29)	9.0	6.4	R_t, PE
2. Camphor (38)	12.0	0.5	R_t, PE
3. Linalyl acetate (9)	16.2	trace	R_t, PE
4. Linalool (2)	11.7	1.2	R_t, PE
5. Dihydrotagetone (14)			NMR
6. (Z)-Ocimenone (30)	18.5	41.0	IR, UV, NMR
7. (E)-Ocimenone (31)	18.2	17.9	IR, UV, NMR
8. Cis-β-Ocimene (11)	11.9	12.1	R_t, PE

Table 3: Identified compounds with their retention times and percentage composition from the oil of TM-F.

Substance	R_t (min)	Percent Composition	Method of Identification
1. Camphor (38)	12.6	2.8	R_t, PE
2. Linalool (2)	11.5	0.2	R_t, PE
3. Myrcene (10)	8.1	trace	R_t, PE
4. Cis-β-Ocimene (11)	11.8	45.7	R_t, IR, UV, NMR
5. (Z)-Ocimenone (30)	20.5	22.0	IR, UV, NMR
6. (E)-Ocimenone (31)	20.2	10.5	IR, UV, NMR
7. Nerol (42)	21.9	0.8	NMR
8. Caryophyllene oxide (41)	30.4	1.7	R_t, PE
9. Humellene (40)	26.9	1.6	R_t, PE

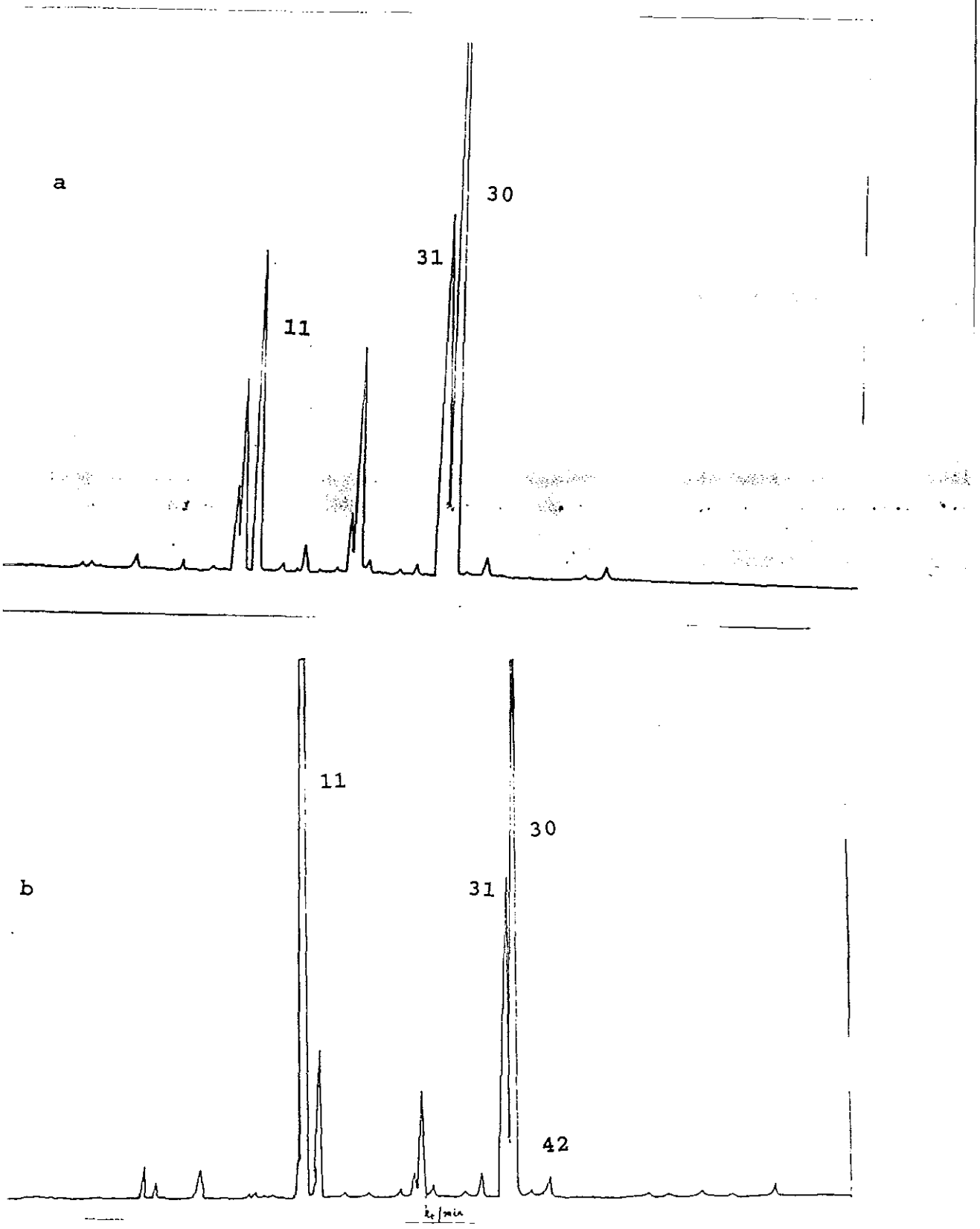

Figure 3. Gas Chromatograms of *T. minuta* a) leaf and b) flower oil.

2.1.1 Isolation and Characterization of *Cis*-β-Ocimene (11)

The slightly yellowish red oil obtained from the flowers of *T. minuta* was applied to a column of silica gel and successively eluted with hexane and EtOAc to separate the hydrocarbon compounds from their oxygenated derivatives. The hydrocarbon fraction TM-F_1 was subjected to GC (*ca.* 60%) and ^{13}C NMR spectroscopy.

Table 4: ^{13}C NMR (22.5 MHz, $CDCl_3$) spectrum of *cis*-β-ocimene **(11)** or TM-F_1

Carbon	δ ppm
1	113.2
2	133.4
3,3-Me	131.7*, 25.4
4	129.4[a]
5	26.3
6	122.4[a]
7,7-Me	131.6*, 19.5[b]
8	17.4[b]

***, a,b: may be interchangeable**

The ^{13}C NMR spectrum of TM-F_1 shows 10 signals (Figure 4). From the DEPT spectrum the peaks at δ 26.3 and δ 113.2 are assignable to methylene groups, the one down field being a terminal methylene. The low intensity peaks at δ 131.7 and δ 131.6 are responsible for quaternary carbons and the three peaks at δ 122.4, δ 129.4, δ 133.4 are to three methine groups. Complete assignment of the ^{13}C NMR spectra of **11** is shown in Table 4. TM-F_1 was further applied to a column of silica gel and eluted using petrol and petrol-EtOAc mixtures. Thirty one fractions each of 10 ml were collected. A colourless fraction TM-F_2 of improved purity (*ca.* 89% on GC) than TM-F_1 was obtained and this pure fraction was subjected to UV and IR analysis. The strong absorption band in the IR spectrum of TM-F_2 at 2900 cm^{-1} is assignable to the carbon-hydrogen stretching frequency for alkane type hydrogens while the weak bands at

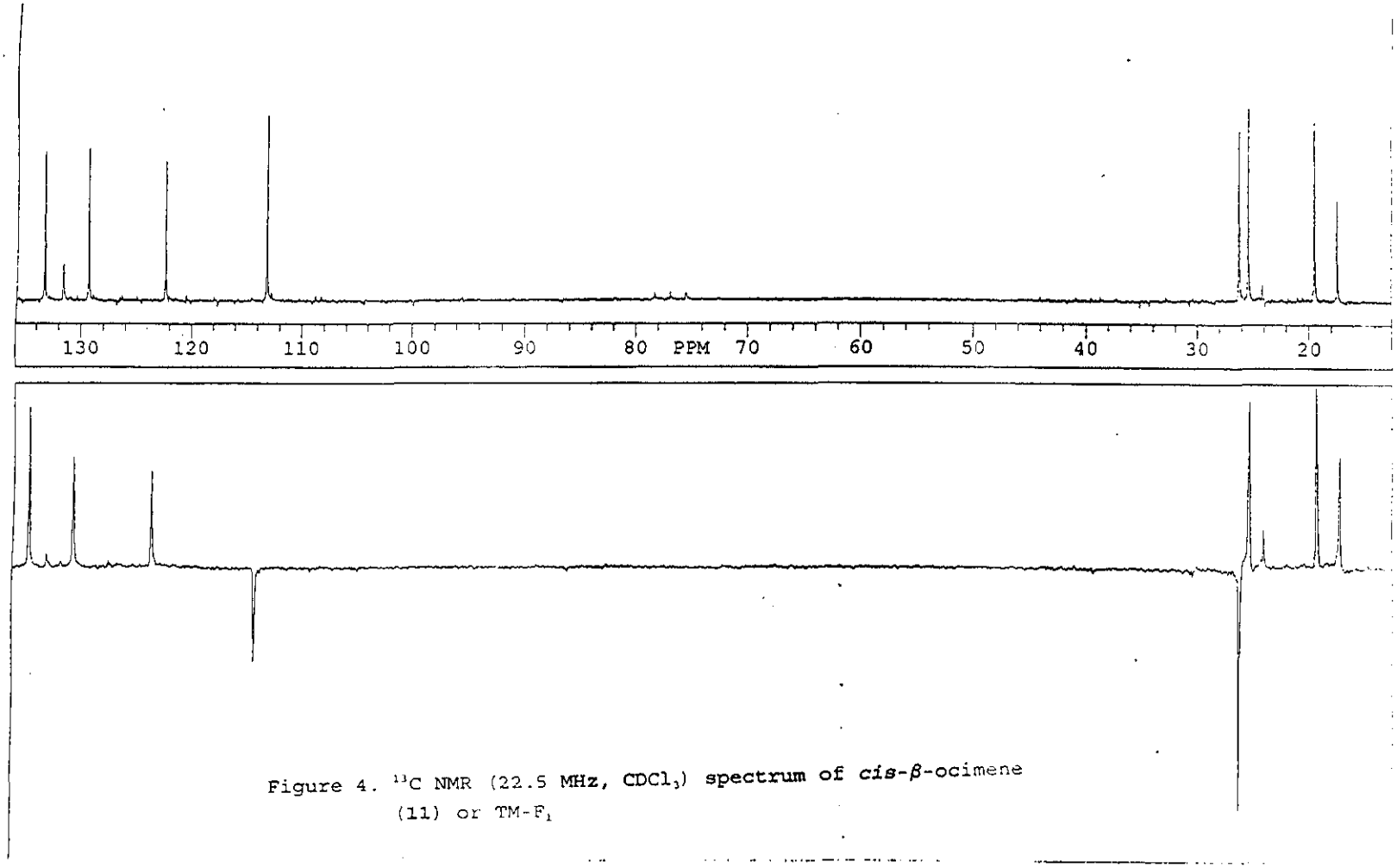

Figure 4. ^{13}C NMR (22.5 MHz, $CDCl_3$) spectrum of *cis*-β-ocimene (11) or TM-F_1

3088 cm^{-1} and 1640 cm^{-1} are attributable to the C-H stretching frequency of vinyl hydrogens and stretching frequency of carbon-carbon double bonds, respectively. From the UV spectrum, the position of the maximum has shifted to a higher wavelength: 238 (λ_{max}), 266, 275 nm. Such bathochromic shift further supported the conjugation and carbon substitution on a diene system.

The peak responsible for the 275 nm may be allo-ocimene (**39**) because ocimene is an unstable compound and when heated it readily isomerises to allo-ocimene (a conjugated triene formed from ocimene by the conjugation of all three double bonds) (Figure 5) which shows an intense maximum in the ultraviolet at 275 nm [57]. The λ_{max} of TM-F_2 is in good agreement with the literature data of *cis*-*β*-ocimene, 237 nm [57]. From all these spectral evidences and comparison with the literature data, the compound was identified as *cis*-*β*-ocimene and it is the major component of the *T. minuta* flower oil.

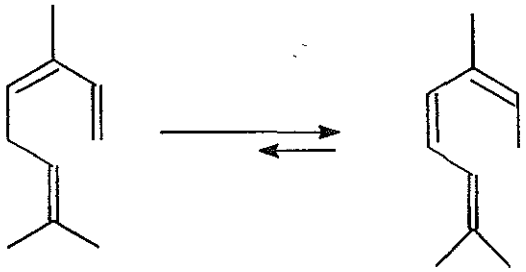

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Figure 5. Isomerization of *cis*-*β*-ocimene (**11**) to allo-ocimene (**39**)

The petrol-EtOAc (8:2) eluted fractions 25-28 and 29-31 were combined separately and were found to contain humellene (**32**) and caryophyllene oxide (**33**), respectively using GC techniques.

2.1.2 Isolation and Characterization of *cis*-Ocimenone (30) and *trans*-Ocimenone (31) from *T. minuta* Flower Oils

The oil of *T. minuta* flower, TM-F was applied to a column of silica gel and eluted with hexane. Successive elution with

EtOAc afforded a yellowish red fraction. This yellowish red fraction was subjected to GC and showed two major peaks. Hence it was further applied to a column of silica gel and eluted with petrol-EtOAc mixtures and 36 different fractions each of 15 ml were collected. Fractions 15-18 (TM-F_3) were combined. The gas chromatogram of fraction TM-F_3 reveals two major components (44%, 48%) with very comparable retention times (20.2, 20.5 min).

Table 5: ^{13}C NMR (22.5 MHz, $CDCl_3$) spectrum of a mixture of *cis*-ocimenone (**30**) and *trans*-ocimenone (**31**) or TM-F_3.

Carbon	*cis*-ocimenone (δ ppm)	*trans*-ocimenone (δ ppm)
1	20.4*	20.6[a]
2,2-Me	155.1[b], 27.8*	155.3[c], 27.8[a]
3	128.2	129.7
4	191.8	191.1
5	140.9	134.9
6,6-Me	148.1[b], 13.2	149.5[c], 13.2
7	126.4	126.1
8	120.6	119.5

*,a,b,c: may be interchangeable

In the analysis of this fraction by ^{13}C NMR spectroscopy, these two components were noted to have also comparable chemical shifts. Close examination of the ^{13}C NMR spectra (Figure 6 and Table 5) led to the conclusion that these two compounds could be geometrical isomers. The absence of $-CH_2-$ and presence of two terminal methylenes (δ 120.6 and δ 119.5), six unsaturated methine carbons (δ 126.1, δ 126.4, δ 128.2, δ 129.7, δ 134.9, δ 140.9), four quaternary carbons (δ 148.1, δ 149.5, δ 155.1, δ 155.3) and two carbonyl carbons (δ 191.8, δ 191.1) suggested that these two compounds are unsaturated carbonyl compounds. This was further confirmed by the strong absorption at 1619 cm^{-1} in the IR spectrum, characteristic of conjugated carbonyl groups. The absorption bands at 2929 cm^{-1} in the IR spectrum indicated the presence of carbon-hydrogen stretching frequency for alkane type hydrogens which are assignable to the methyl

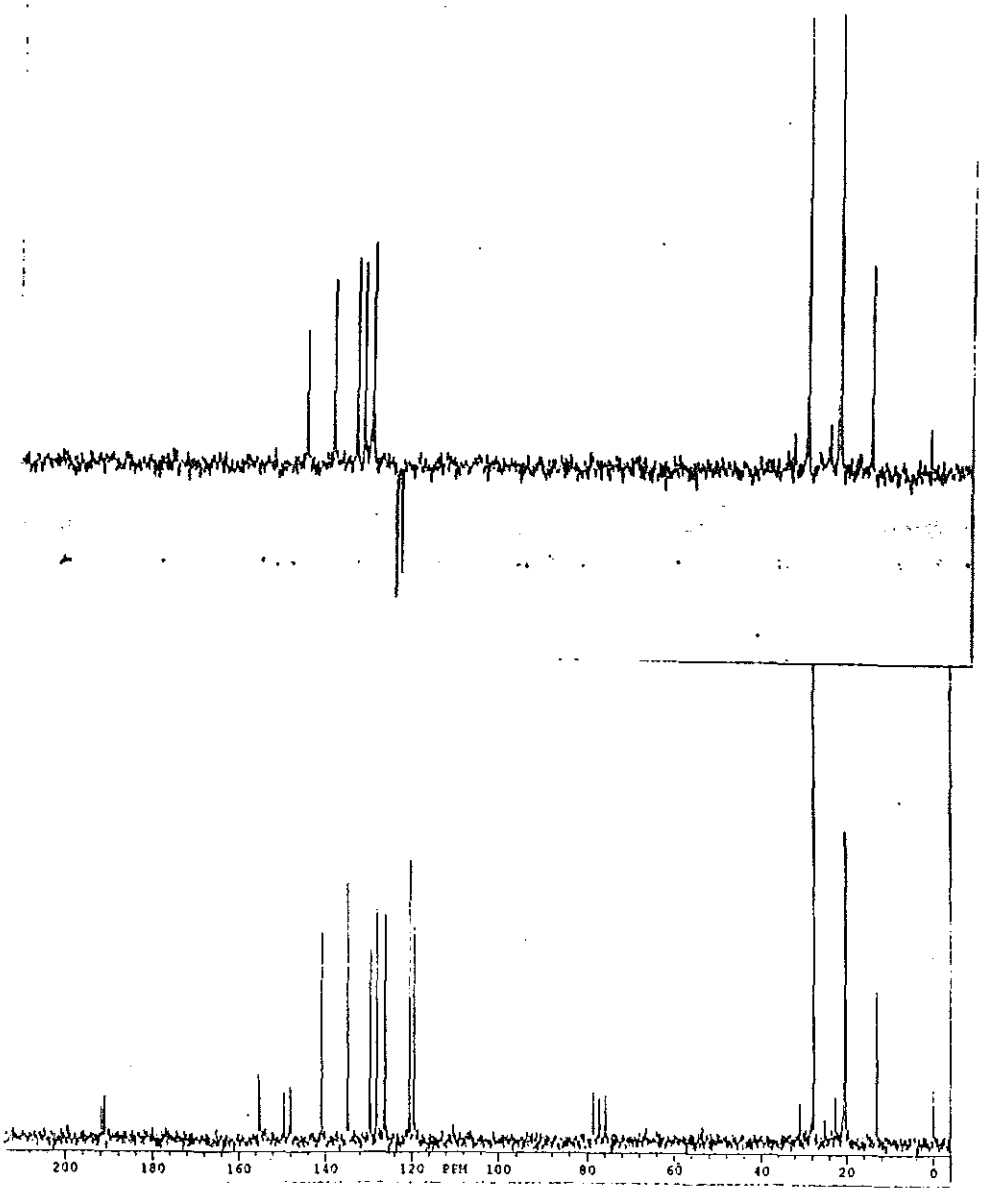


Figure 6. ^{13}C NMR (22.5 MHz, $CDCL_3$) of a mixture of *cis*-ocimenone (30) and *trans*-ocimenone (31) or TM-F_3.

substituents. Despite their comparable concentrations, only one λ_{max} (288 nm) in the UV spectrum was observed and such a shift to a longer wavelength is indicative of conjugation and carbon substitution on a diene system. The above spectral evidences and their close boiling points led us to conclude the two components could be geometrical isomers: (Z)-ocimenone (30) and (E)-ocimenone (31). Based on these observations it is the *cis* isomer which is present in larger (22.0%) and the *trans* in smaller (10.5%) amounts in the crude *T. minuta* flower oil.

2.1.3 Isolation and Characterization of Nerol (42)

The flower oil of *T. minuta*, TM-F was applied on a column of silica gel and eluted with hexane. Successive elution with EtOAc afforded a yellowish red oil. This yellowish red fraction was further applied to a column of silica gel and eluted with petrol-EtOAc mixtures and different fractions each of 15 ml were collected. Fractions 23-35 (coded as $TM-F_4$) were combined based on their R_f value. $TM-F_4$ was subjected to GC, ^1H and ^{13}C NMR analyses.

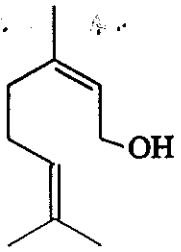
Table 6: ^1H NMR (90 MHz, $CDCl_3$) spectrum of nerol (42) or $TM-F_4$.

Hydrogen	δ ppm
1, 1-OH	4.85 (2H,dd), 2.95 (1H,t)
2	5.90 (1H,m)
3-CH_3	1.25 (3H,d)
4	2.00 (2H,m)
5	2.25 (2H,m)
6	6.10 (1H,m)
7-CH_3	1.70 (3H,d)
8	1.95 (3H,d)

Table 7: ^{13}C NMR (22.5 MHz, $CDCl_3$) spectrum of nerol (**42**) or TM-F_4.

Carbon	δ ppm
1	53.7
2	126.6
3, 3-Me	162.0, 20.5
4	30.2*
5	29.5*
6	113.5
7, 7-Me	143.0, 24.1
8	27.6

*:may be interchangeable



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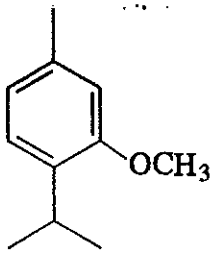

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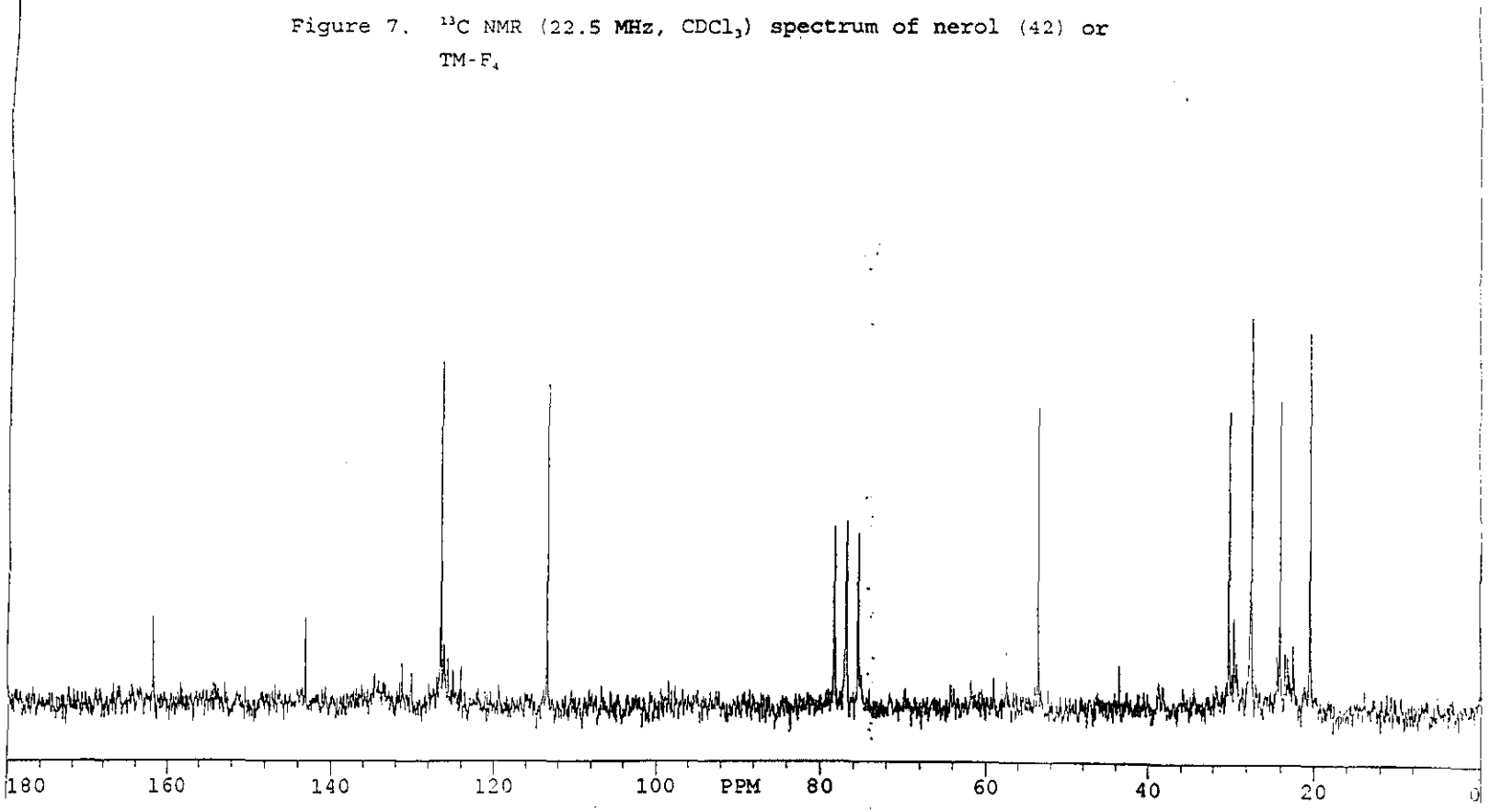
The ^{13}C spectrum of TM-F_4 showed 10 carbon signals (Figure 7). The peak at δ 53.7 is characteristic of methoxy (-OCH_3) carbon and this suggested a thymolmethyl ether (**43**). But the absence of signals for -OCH_3 and aromatic protons in the ^1H NMR spectrum at their characteristic regions ruled out the possibility of being thymolmethyl ether (**43**). Thus the signal at δ 53.7 in the ^{13}C NMR spectrum is that of a carbon attached to an electron withdrawing atom. The signals at δ 162.0 and δ 143.0 are responsible for two quaternary carbons (C-3 and C-7). C-3 being down field than C-7 due to the electron withdrawing effect of the oxygen atom. The doublet at δ 1.25 in the ^1H NMR spectrum is assignable to the C-3 methyl protons which are coupled with H-2 (allylic coupling). The doublets with small

Figure 7. ^{13}C NMR (22.5 MHz, $CDCl_3$) spectrum of nerol (42) or TM-F_4



coupling constants at δ 1.70 and δ 1.95 are due to the two methyl protons (7-CH_3 and H-8). The multiplets at δ 2.00 and δ 2.25 (which may be interchangeable) are assignable to H-4 and H-5, respectively (for complete assignment of the ^1H and ^{13}C NMR spectra, See Tables 6 and 7). These spectral data suggest this substance to be nerol (**42**), in good agreement with the literature [58,59].

2.1.4 Isolation and Characterization of Dihydrotagetone (14)

The leaf oil of *T. minuta* (TM-L) was separated in to its hydrocarbon and oxygenated fractions by eluting with hexane and EtOAc, respectively. The hexane fraction was further applied to a column of silica gel and eluted by increasing the polarity from petrol to EtOAc. The progress of the separation was monitored by TLC. Different fractions each of 10 ml were collected and fractions containing compounds with the same R_f were combined. Fractions 14-25 (coded as TM-L_1) eluted with petrol were combined and removal of the solvent afforded 73.9 mg TM-L^1. This compound was subjected to GC and NMR analyses, and was identified as dihydrotagetone (**14**).

Table 8: ^{13}C NMR (22.5 MHz, $CDCl_3$) spectrum of dihydrotagetone (**14**) or TM-L_1

Carbon	δ ppm
1	22.5
2,2-Me	33.1[b], 22.5
3	49.8*
4	209.9
5	52.4*
6, 6-Me	24.4[b], 19.7
7	142.9
8	112.9

*,[b]: may be interchangeable

The ^{13}C NMR spectrum of TM-L_1 shows signals for 10 carbons (Figure 8). The small intensity signals at δ 24.4 and δ 33.1

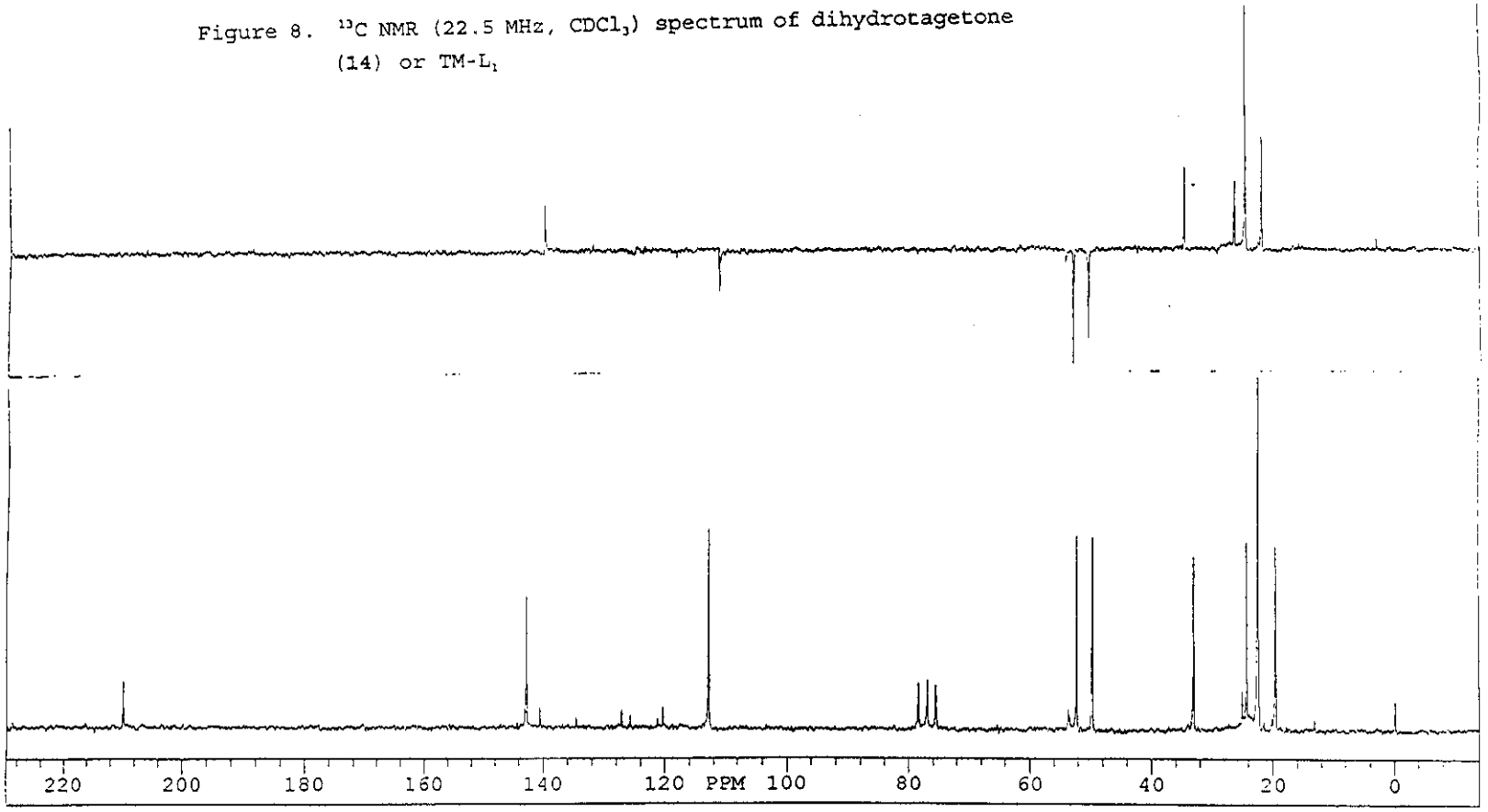
are assignable to C-2 and C-6 which may be interchangeable. The up field signals at δ 19.7, δ 22.5 are due methyl substituents. The peak at δ 209.9 is characteristic of carbonyl carbon (does not appear in the DEPT spectrum). From the DEPT spectra three methylene groups at δ 112.9, δ 52.4 and δ 49.8 were observed. The one down field (δ 112.9) being a terminal methylene. The peak at δ 142.9 is responsible for one unsaturated methine (=CH-) group. Hence the compound has got only one carbon carbon double bond and a carbonyl carbon. With the help of these spectral evidences the compound was identified as dihydrotagetone (**14**) (for complete assignment of the ^{13}C spectra, See Table 8).

2.1.5 Isolation and Characterization of *cis*-ocimenone (30) and *trans*-ocimenone (31) from *T. minuta* Leaf Oil.

The oil of *T. minuta* leaf, TM-L was applied on a column of silica gel and eluted with hexane. Successive elution with EtOAc afforded a yellowish red fraction. This yellowish red fraction was further run on a column of silica gel and eluted by increasing the polarity from petrol to EtOAc (see Experimental part). The separation of the component on the silica gel was clearly observed as a deep yellowish band separated from the other components. The progress of the separation was controlled by TLC and fractions containing compounds with the same R_f were combined. 70 fractions each of 10 ml were collected. Fractions 7-30 eluted with petrol were combined and removal of the solvent afforded 700 mg of TM-L_2. This was subjected to $_{13}C$ NMR, UV and IR analyses and was identified as a mixture of two geometrical isomers: (Z)-ocimenone (**30**) and (E)-ocimenone (**31**). Essentially the same deep red colour, R_t, λ_{max}, ν_{max} and similar ^{13}C spectra was observed for TM-F_3 and TM-L_2 (See Table 5 and Experimental part). Hence, the two major components of this fraction are the two geometrical isomers identified in TM-F_3. These two geometrical isomers: (*Z*)-ocimenone (**30**) and (*E*)-ocimenone (**31**) are the major components of *T. minuta* leaf oil.

The characteristic deep red colour of TM-F_3 and TM-L_2 is

Figure 8. ^{13}C NMR (22.5 MHz, $CDCl_3$) spectrum of dihydrotagetone (14) or TM-L_1



responsible for the reddish yellow colour of *T. minuta* oils, and that is why the *T. minuta* leaf oils are more deep yellowish red than the flower oils. Upon exposure to air the oils of *T. minuta* turn thick but the degree of thickening is greater in the leaf oils where the composition of the two geometrical isomers is high. Therefore, *cis*- and *trans*-ocimenones may be responsible for such property of these oils.

2.1.6 Comparison between Leaf and Flower Oils of *T. minuta*

The variation in composition in different parts of a plant is well known. There is marked difference between the oils

Table 9: Comparison of the major components of *T. minuta* oil [26, 60].

OILS	C O M P O U N D S					
	11	**12**	**13**	**14**	**30**	**31**
1	40.7	5.4	4.7	5.1	1.1	0.5
2	48.2	7.0	7.7	13.0	2.3	1.6
3	54.5	6.1	4.4	13.5	1.3	1.3
4	27.9	5.0	4.7	20.0	0.3	0.6
5	44.3	6.6	2.4	5.3	7.6	1.1
6	36.8	t	12.1	18.3	15.5	5.7
7	42.9	t	10.9	12.0	16.2	5.3
8	40.0	t	4.6	16.4	13.1	4.0
9	45.7				22.0	10.5
10	12.1				41.0	17.9

Legend: 1-5 Commercial oils
6-7 North American Produced Oils
8 Brazilian Oil
9 Ethiopian Flower Oil
10 Ethiopian Leaf Oil, t Trace

obtained from the flowers and leaves of *T. minuta* of Ethiopian origin. The major component of the flower oils is *cis*-ocimene while that of the leaf oils are the oxygenated *cis*- and *trans*-ocimenones. Commercially this variation could be important.

Table 9 clearly indicates that the flower oils obtained from *T. minuta* investigated in this study have got a better potential for commercial purposes than the leaf oils even though some of the remaining components of the oils must be identified.

2.2 *Artemisia rehan Chiov.*

Aerial parts of *A. rehan* were purchased from street markets in December, February and March, 1995 and subjected to steam distillation. The oils were coded as AR-1 (December), AR-2 (February) and AR-3 (March) and obtained in 0.27%, 0.38% and 0.78% yield (fr. wt.), respectively. They do posses a characteristic pleasant smell and deep blue colour. This deep blue colour hindered the measurement of optical rotation and refractive index. The gas chromatograms of AR-1, AR-2 and AR-3 obtained by running on DB-5 non polar capillary column revealed 41, 40 and 41 components, respectively (Figure 9). Comparison of retention times with standard samples, peak enhancement experiments, ^{1}H and ^{13}C NMR analyses on these oils resulted in the identification of the compounds shown in Table 10.

Table 10: Compounds identified from *A. rehan* oils purchased at different times.

Substance	AR-1 (%)	AR-2 (%)	AR-3 (%)	Method
1. Camphor (38)	5.2	30.4	31.1	R_t, PE
2. Linalool (2)	2.1	5.1	3.9	R_t, PE
3. γ-Terpinene	0.5	1.3	1.7	R_t, PE
4. 1,8-Cineole (29)	trace	0.4	1.1	R_t, PE
5. Myrcene (10)	0.6	2.7	2.8	R_t, PE
6. Chamazulene (33)	7.6	1.9	1.0	NMR*

*: Characterized from AR-1.

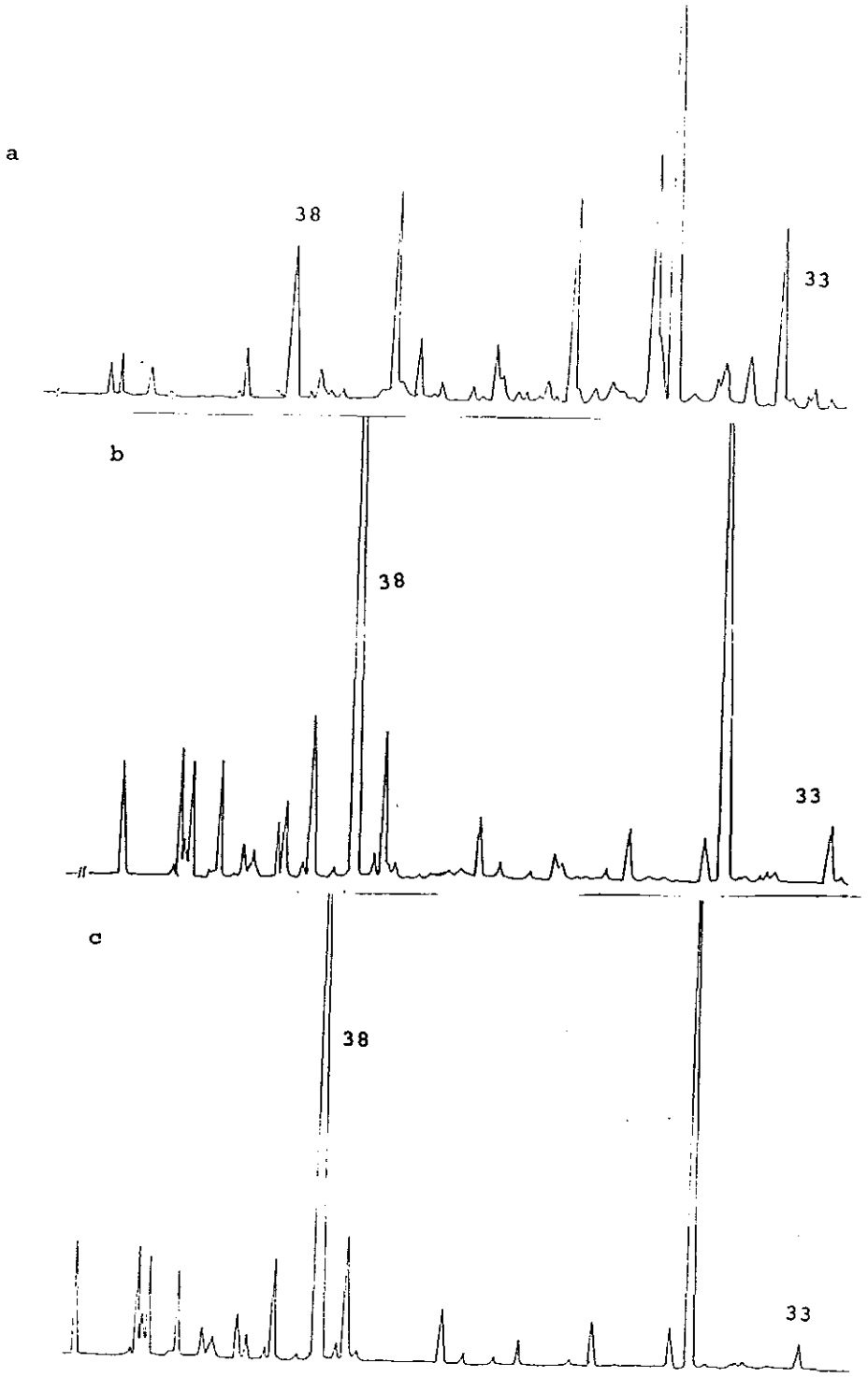


Figure 9. Gas Chromatograms of *A. rehan* samples purchased at different times. a) AR-1, b) AR-2, c) AR-3

The oils isolated from the aerial parts of *A. rehan* purchased at different times from street markets show remarkable variations in their component compositions (Figure 9) and yields which increased from December to February and then to March. Previous works with the December oils of *A. rehan* [9] from Ethiopia indicated camphor to be the second major component while in the December oils investigated in this study, camphor was found as a minor component (5.2%). But gas chromatograms of the oils from the samples collected in February and March clearly showed camphor to be the major component. But the composition of chamazulene decreased from December to March, though its variation is not significant as that of camphor. The marked difference between the oils AR-1, AR-2 and AR-3 in yield and component composition may be due to seasonal variation, existence of different chemotypes of *A.rehan* or difference in stages of development of the plant materials.

2.2.1 Isolation and Characterization of Chamazulene (33)

The blue oil (AR-1) obtained from the above ground part of *A. rehan* was run on a column of silica gel and eluted with hexane. Seven hexane and three EtOAc fractions each of 50 ml were collected. The separation of the blue component (hexane eluted) on the silica gel was clearly observed as a blue band separated from the other components. The 3rd and 4th hexane eluted fractions (blue colored) were combined, concentrated and subjected to GC, ^{1}H and ^{13}C NMR analyses. The compound was identified as chamazulene (**33**) on the basis of its ^{1}H and ^{13}C NMR spectra and these were found to be in good agreement with the literature [61].

The doublet at δ 8.2 in the ^{1}H NMR spectrum (Figure 10) of chamazulene is assigned to the H-8 proton which is coupled to the H-6 (J= 1.8 Hz, allylic coupling). This serves to identify the H-5 at δ 7.0 ($J_{5,6}$= 9 Hz) and the H-6 at δ 7.4 ($J_{6,5}$= 9 Hz, $J_{6,8}$= 1.8 Hz). Coupling constants in five membered rings are approximately equal and small. Hence, the doublet at δ 7.65 ($J_{2,3}$= 2.25 Hz) and δ 7.25 ($J_{3,2}$= 2.25 Hz) are assigned to H-2

and H-3, respectively.

Positions 1 and 3 have got greatest excess π electron charge density [61], that is why the signal due to H-2 is down field than H-3. The ^{13}C NMR spectrum revealed 9 signals (Figure 10). The remaining quaternary carbons were not observable. The signals at δ 12.8, δ 17.2 and δ 24.0 are assignable to the three methyl groups. From the DEPT spectrum the signal at δ 33.8 is due to the methylene group on C-7 and the down field signals at δ 112.8, δ 124.8, δ 134.5, δ 136.2 and δ 137.4 are assignable to the five methine groups (for complete assignment of the ^{1}H and ^{13}C NMR see Tables 11 and 12).

Table 11: ^{1}H NMR (90 MHz, $CDCl_3$) spectrum of chamazulene (33).

Hydrogen	δ ppm
1-CH_3	2.85 (3H, s)
2	7.65 (1H, d, $J_{2,3}$=2.25 Hz)
3	7.25 (1H, d, $J_{3,2}$=2.25 Hz)
4-CH_3	2.65 (3H, s)
5	7.00 (1H, d, $J_{5,6}$=9 Hz)
6	7.40 (1H, dd, $J_{6,8}$=1.8 Hz)
7-CH_2-	2.90 (2H, q)
7-CH_3	1.35 (3H, t)
8	8.20 (1H, d, $J_{8,6}$=1.8 Hz)

Table 12: ^{13}C NMR (22.5 MHz, $CDCl_3$) spectrum of chamazulene (33).

Carbon	δ ppm
1-Me	12.8
2	124.9
3	112.8
4-Me	17.2
5	134.5
6	136.2
7-CH_2, CH_3	33.8, 24.0
8	137.4

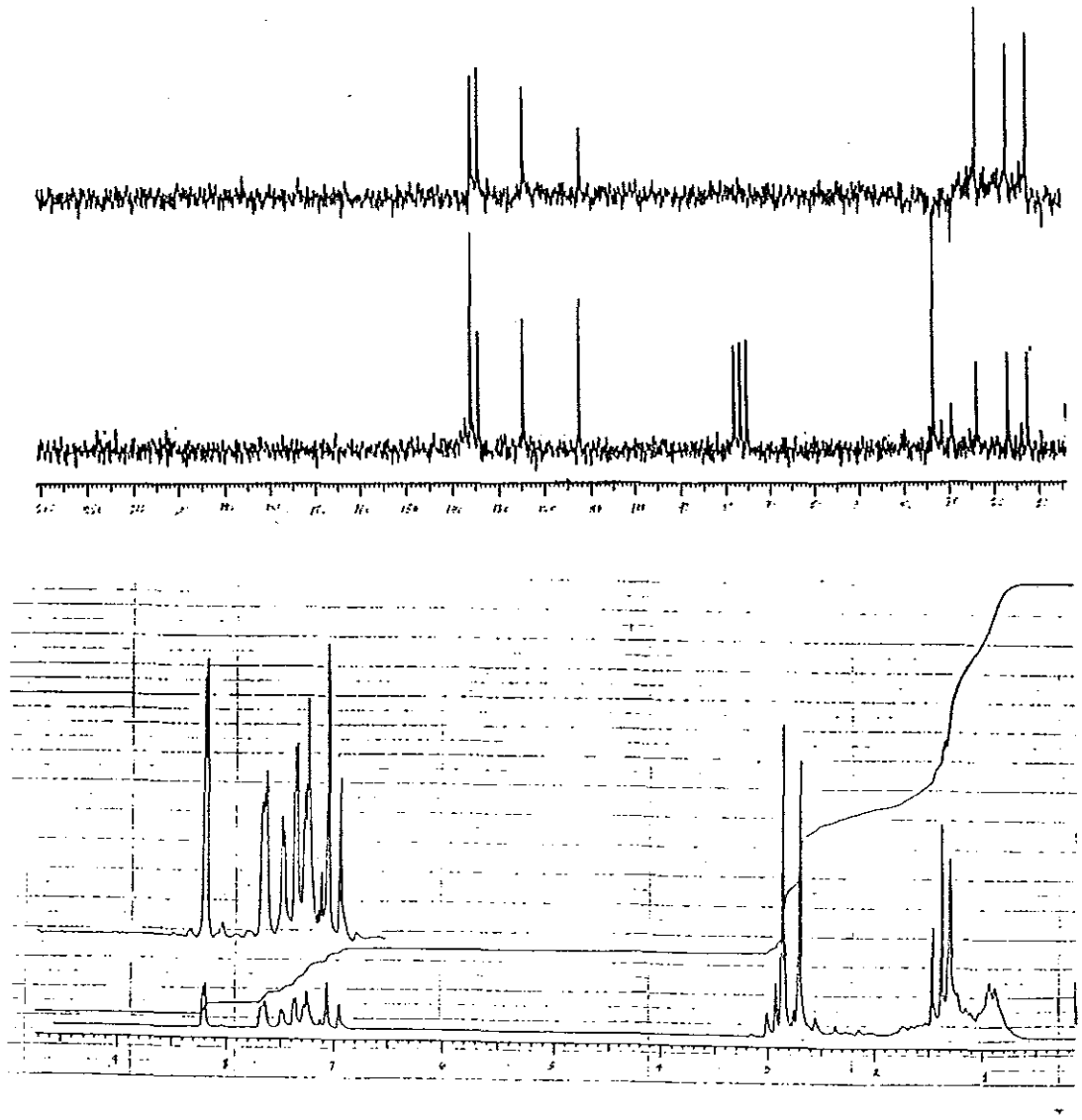


Figure 10. ^{1}H and ^{13}C NMR spectrum of chamazulene (33)

This blue component is most likely an artifact obtained from sesquiterpenoid lactones such as artabsin **44** (a substance isolated from wormwood), or matricin **45** (a substance isolated from camomile) as a result of dehydration, decarboxylation and/or deacetylation [9]. The formation of chamazulene during steam distillation of other plant materials such as *A. caruthii* [62] has been reported. It has also been reported that matricin is concentrated in the bitter leaves of the plant and exhibits its maximum concentration during July and almost undetectable by September in the plant. Search for the precursors of Chamazulene (pro-chamazulenogens) led to the isolation of **44** and **45** which upon heating in slightly acidic media gave the blue compound chamazulene [54]. Chamazulene is formed from matricin by a series of reactions on mere boiling with water, while artabsin gives first the yellow orange dihydrochamazulene (**46,47**) which is then oxidized by air to the blue hydrocarbon [54] (Figure 11).

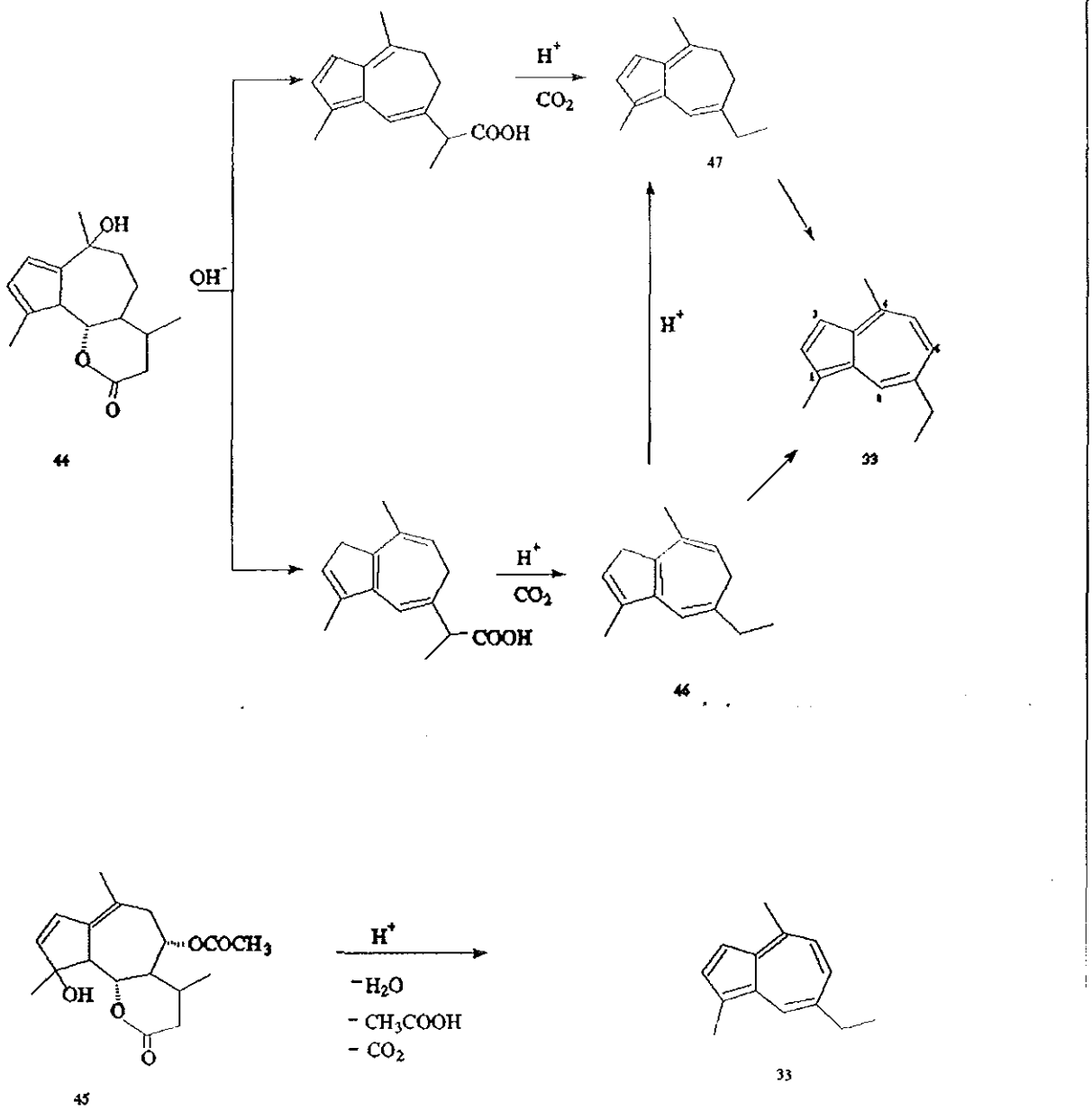


Figure 11: Formation of chamazulene from artabsin and matricin.

3. CONCLUSION AND RECOMMENDATIONS FOR FUTURE WORK

In this work the main components of the leaves and flowers of *T. minuta* of Ethiopian origin have been analyzed for the first time. The flowers were found to have better potential for commercial purposes than the leaf oils. Comparison of the above ground parts, seeds and root oils of *T. minuta* with the commercial oils is recommended for the future. Basically this work is a value added research. Therefore, it could be used as base line data to determine the potential of *T. minuta* for commercial scale production of essential oils in this country.

Variation in composition and yield of the essential oils of *A. rehan* purchased at different times have been observed. Chamazulene is believed to be an artifact which is formed from sesquiterpenoid lactones such as artabsin and matricin during steam distillation. Hence further work is recommended to

- Identify the cause for the remarkable variation in yield and component composition on garden grown samples.
- Isolate the precursors of chamazulene and confirm whether it is an artifact or a natural product.

Likewise, it is hoped that this work will contribute for the commercial exploitation of *A. rehan* essential oils.

4. EXPERIMENTAL

General. Neat optical rotations were measured using Perkin Elmer polarimeter model 241. Refractive indices were determined on Abbe's refractometer. Specific gravity of the oils was determined using a pycnometer. Analytical TLC were run on a 0.25 mm thick layer of silica gel GF_{254} (Merck). Spots were detected by observation under UV light (254 nm) and spraying with 1% vanillin H_2SO_4 and heating. Flash column chromatography was performed using silica gel 60 (230-400 mesh) Merck. Gas chromatographic analysis of the oils, fractions, standard samples and mixtures was performed on Varian model 3700 gas chromatograph equipped with flame ionization detector (FID) using DB-5 non polar capillary column with 30 m x 0.23 mm (i.d.) and 1.5 micron film thickness. Analyses were performed by programming the column temperature from 60 to 190°C at 4°C min^{-1}. Injector and detector temperatures were 220 and 270°C, respectively. N_2 at 25 ml min^{-1} was used as a carrier gas. Peak areas were determined with the aid of a Varian 4290 integrator. Standard mixtures were prepared from standard samples which were obtained from Essential Oils research and Development Centre (EORC) and research laboratories of the Chemistry Department, AAU. Samples of 0.02 μl were injected.

^1H and ^{13}C NMR spectra in $CDCl_3$ were recorded on a Joel FX 90Q at 90 MHz and 22.5 MHz, respectively and are reported as ppm values relative to TMS internal standard. Neat IR spectra of the crude and fractionated oils were recorded using the Perkin Elmer FTIR spectrometer model 1600 with NaCl cell. UV-VIS spectra were recorded using Milton spectronic 1001 plus spectrophotometer.

Plant Materials:

Tagetes minuta L. (S-761) was collected from Kottebe (around the bridge near Wondirad Sec. School) during flowering stage. *Artemisia rehan Chiov.* (S-805, S-818,S-821) samples were collected from Addis Ababa and Sebetta markets. The plant materials were identified by Dr. Sebsibe Demissew and voucher

specimens (depicted in brackets) have been deposited at the National Herbarium of Addis Ababa University.

Extraction: The leaves and flowers of *T. minuta* and above ground parts of *A. rehan* purchased at different times were comminuted to suitable size and subjected to steam distillation. Each batch was steam distilled for about 3-3:30 hr. All the oils were dried over Na_2SO_4.

4.1 Isolation and Identification of Compounds from *T. minuta*

Flower oils: 3 g of the flower oil was applied on 45 g of column silica gel. This was eluted with 300 ml of distilled n-hexane followed by 200 ml of EtOAc. The hexane fraction, TM-F_1 and the oxygenated (EtOAc) fraction were subjected to GLC analysis after the solvents were removed by rotary evaporator. 900 mg of the hexane fraction was obtained and analyzed by GC (*ca.* 60%) and ^{13}C NMR. To improve the purity of the hexane fraction, it was further applied to a column of silica gel. The petrol eluted fractions (of improved purity *ca.* 89%) were then subjected to IR and UV analysis. Compound **11** was isolated from TM-F_1.

Cis-β-Ocimene (**11**). $C_{10}H_{16}$; Colour: Colourless; UV-VIS λ_{max} (MeOH) nm: 238 (max), 266, 275; GC R_t min: 11.0; IR ν_{max} (neat) cm^{-1}: 3407.5, 3088.8, 2926.1, 2856.8, 1726.3, 1681.1, 1639.7, 1445.9, 1378.2, 1320.0, 1256.9, 1156.6, 1109.6, 986.0, 902.0, 831.2; ^{13}C NMR: See Table 4.

The oxygenated fraction was further applied on a column of silica gel and eluted by increasing the polarity from petrol to petrol-EtOAc (98:2, 95:5, 9:1, 8:2) and EtOAc. 36 different fractions each of 15 ml were collected. Based on their R_f (solvent system: petrol-Et_2O, 9:1) fractions 15-18 (TM-F_3) and 23-25 (TM-F_4) were combined separately and concentrated. A mixture of compounds **30** and **31** were isolated from TM-F_3 and compound **42** from TM-F_4.

Mixtures of *cis*-ocimenone (**30**) and *trans*-ocimenone (**31**). $C_{10}H_{14}O$; Colour: Deep red; GC R_t min: 20.5 and 20.2 respectively; UV-VIS λ_{max} (MeOH) nm: 288.7; IR ν_{max} (neat) cm^{-1}: 3450.8, 3092.0, 2929.8, 2750.0, 1665.9, 1619.9, 1582.8, 1443.7, 1380.5, 1225.0, 1115.8, 1073.9, 1044.4, 990.0, 920.5, 878.9, 758.3; ^{13}C NMR: See Table 5.

Nerol (**42**). $C_{10}H_{18}O$; Colour: Light yellow; ^{1}H NMR: See Table 6; ^{13}C NMR: See Table 7.

Leaf oils: 3 g of *T. minuta* leaf oils were applied to a column of silica gel (45 g) and eluted with 300 ml of distilled hexane followed by 200 ml of EtOAc. 600 mg of hexane fraction and 1.8 g of the oxygenated fraction were obtained. All of the hexane fraction were further applied on a column of silica gel and eluted by increasing the polarity from petrol to petrol-EtOAc (98:2, 95:5, 4:1) and EtOAc. The petrol eluted fractions 14-25 ($TM-L_1$) having the same R_f value (solvent system: petrol-$CHCl_3$, 1:1; C_6H_6-petrol-EtOAc, 3:9:1) were combined and subjected to GC and ^{13}C NMR. Compound **14** was isolated from $TM-L_1$.

Dihydrotagetone **14**. $C_{10}H_{18}O$; Colour: Light yellow; ^{13}C NMR: See Table 8.

1.8 g of the oxygenated fraction were further applied on a column of silica gel and eluted with petrol, petrol-EtOAc (95:5, 9:1, 4:1, 1:1) and EtOAc. 70 fractions each of 10 ml were collected. The petrol eluted fractions 7-30 ($TM-L_2$) having the same R_f value (solvent system: petrol-Et_2O, 9:1) were combined and subjected to GC, UV, IR and ^{13}C NMR. A mixture of compounds **30** and **31** was isolated from $TM-L_2$.

Mixture of *cis*-ocimenone (**30**) and *trans*-ocimenone (**31**). $C_{10}H_{14}O$; Colour: Deep red; UV-VIS λ_{max} (MeOH) nm: 289.0; IR ν_{max} (neat) cm^{-1}: 3463.3, 3092.3, 2928.9, 2728.8, 2364.7, 2182.8, 2055.0, 1845.9, 1665.8, 1620.7, 1579.4, 1444.0, 1380.8, 1290.4, 1226.1, 1115.0, 1043.0, 1002.3, 920.7, 876.6, 767.4; ^{13}C NMR: See Table 5.

4.2 Isolation and Identification of Compounds from *A. rehan*

From 1470 (December), 1300 (February) and 540 (March) g of purchased above ground parts of *A. rehan* 4, 5 and 4.2 ml steam distillate were obtained, respectively. 2.5 g of the oil of *A. rehan* purchased in December was applied on 40 g of silica gel. This was eluted with hexane and EtOAc. Seven hexane and three EtOAc fractions were obtained. The 3rd and 4th fractions of the hexane eluents were combined, concentrated and subjected to GC, ^{1}H and ^{13}C NMR. Compound **33** was isolated from this fraction.

Chamazulene (33). $C_{14}H_{16}$; Colour: deep blue; GC R_t min: 29.9; ^{1}H NMR: See Table 11; ^{13}C NMR: See Table 12: quaternary carbons were not detected.

Camphor (38). $C_{10}H_{16}O$; GC R_t min: 12.9; Camphor was identified by a direct GC comparison with authentic sample.

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D E C L A R A T I O N

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

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This thesis has been submitted for examination with my approval as a university advisor.

Dr Ermias Dagne _____