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Gas Liquid Chromatographic Researches on the Volatile Oil of a Thymus Species (Thymus Sipyleus Boiss.)With a Lemon - like Odour

Limon Kokulu Bir Kekik Türünün (Thymus sipyleus Boiss.) Uçucu Yağında Gaz Kromatografisi ile Araştırmalar

Nevin TANKER (*)

Near south of Ankara, on 40 th km of the Ankara-Bâlâ highway, there is a forest named "Beynam Forest". The flora of this forest is represented by 419 species and one of them is *Thymus sipyleus* Boiss. which has been found in calcareous steppe at about 1300 m height (1).

The genus *Thymus* (Labiatae) is characterized by its thymol odour. Although, there are some *Thymus* species such as *T. citriodorus* Schreb., *T hirtus* Viv. and *T. hyemalis* Lange, they do not have the same odour. They smell like lemon (2).

Thymus sipyleus Boiss. which was collected from Beynam Forest (Ankara) has the morphological characters similar to that of *Thymus serpyllum L. (= T. jankae* (3,4)) but it differs from this species in having rose flowers and lemonlike odour (**).

EXPERIMENTAL

MATERIAL and METHOD

The main purpose of the work presented here is to find out the compounds which give the plant its characteristic odour.

The plant material was collected in May, June and July. Aged and dry stems were cut off and fresh parts were used.

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(**) This plant is determined by Jaakko jalas - Helsinki.

Isolation of volatile oil :

Fresh stems, leaves and flowers were cut into small pieces with a knife, were mixed with water and then the essential oil was distilled in a Clevenger apparatus. The isolated oil is dried over anhydrous Na_2SO_4, and yield is 0.5 %.

The volatile oil is yellow and it has a strong cineol - citral odour.

$d_{20} = 0.9216$
$n_{20} = 1.4840$
$[\alpha] D_{20} = -27.7°$

Separation of the volatile oil :

Because of the complex nature of volatile oils, it is often desirable to separate the compounds into groups prior to the gas chromatographic analysis.

A volatile oil can be separated into different fractions by means of different chromatographic techniques and each fraction can be examined apart by means of gas liquid chromatography. In this way a better separation of the components can be obtained and thus the peaks on the chromatograms obtained by gas chromatography can be identified easily (5, 6). The low boiling monoterpene hydrocarbons and the higher boiling oxygen containing monoterpenes were separated from each other by using the method recommended by KARLSEN and Co. (6).

Separation of the monoterpene hydrocarbon fraction :

20 gram of silica gel (Kieselgel 0.05 - 0.2 mm for column chromatography) in 50 ml n - pentane (boil. range 34 - 37°C) was transferred to a chromatographic column, 1 cm diameter. After draining of the pentane, 1 ml of isolated oil was added on the top of the column. Then, terpene hydrocarbons were eluted with n - pentane The solvent was evaporated carefully on a water bath and monoterpene hydrocarbon mixture was obtained.

Separation of the oxygenated compounds :

After removing all the monoterpene hydrocarbons, the column was eluted with ethyl acetate. By evaporating the solvent carefully on a water bath, the higher boiling oxygen containing monoterpenes were obtained.

Gas liquid chromatography of the monoterpene hydrocarbons :

In order to obtain good separated peaks on the gas chromatograms, several stationary phases were tested. The most suitable temperature, gas pressure and the packing material were choosen.

Gas liquid chromatograph

An Aerograph Model 1520 and Packard Model 409 Becker Gas Chromatograph, each equipped with hydrogen flame detectors were used for the experimental work.

System I

Gas chromatograph	:	Aerograph 1520
Detector	:	FID (flame ionization detector)
Column	:	Copper coil, 8 m long, inner diameter 1.5 mm
Solid support	:	Chromosorb W 60/80 mesh, acid washed
Stationary phase	:	Carbowax 1540 10 %
Temperature	:	70°C isothermal, (detector 225°C, injector 200°C)
Carrier gas	:	Nitrogen
Flow rate	:	30 - 35 ml/min.

System II

Gas chromatograph	:	Packard 409
Detector	:	FID
Column	:	Copper coil, 8 m long, inner diameter 1.5 mm
Solid support	:	Chromosorb W 60/80 mesh, acid washed
Stationary phase	:	β, β - Oxydiproprionitrile 10 %
Temperature	:	30°C isothermal (detector 180°C, injector 190°C)
Carrier gas	:	Nitrogen
Flow rate	:	30 ml/min.
Inlet pressure	:	4.5 kp/cm²

Gas liquid chromatography of the oxygenated compounds :

System III

Gas chromatograph	:	Packard 409
Detector	:	FID
Column	:	Copper coil, 8 m long, inner diameter 1.5 mm
Solid support	:	Chromosorb W 60/80 mesh, acid washed
Stationary phase	:	PEG 20M (polyethylene glycol)
Temperature	:	140°C isothermal
Carrier gas	:	Nitrogen
Flow rate	:	4.6 kp/cm², 6 kp/cm²

System IV

Gas chromatograph	:	Aerograph 1520
Detector	:	FID
Column	:	Copper coil, 8 m long, inner diameter 1.5 mm
Solid support	:	Chromosorb W 60/80 mesh, acid washed, silan
Stationary phase	:	Carbowax 1540 10 %
Temperature	:	125°C isothermal (detector 240°C, injector 180°C)
Carrier gas	:	Nitrogen
Flow rate	:	30 ml/min.

Direct gas liquid chromatography of plant material :

The volatile oils which are used for the gas chromatographic studies have been isolated from the plant material, by means of steam distillation. During the distillation some of the components are decomposed. Therefore the gas chromatographic results may not show the correct composition of the essential oil in the living plant. For this reason Baerheim SVENDSEN and KARLSEN made researches and found out a new technique for the analysis of lower terpenens in plant material (7,8). This solid sampling technique allows the plant material to be brought into the heated injector zone of the gas chromatograph where the volatile compounds distill off into the column. This technique provides the qualitative and quantitative analysis of terpenens in plant (9). No modification of the injection port of the gas chromatograph is necessary (10, 11).

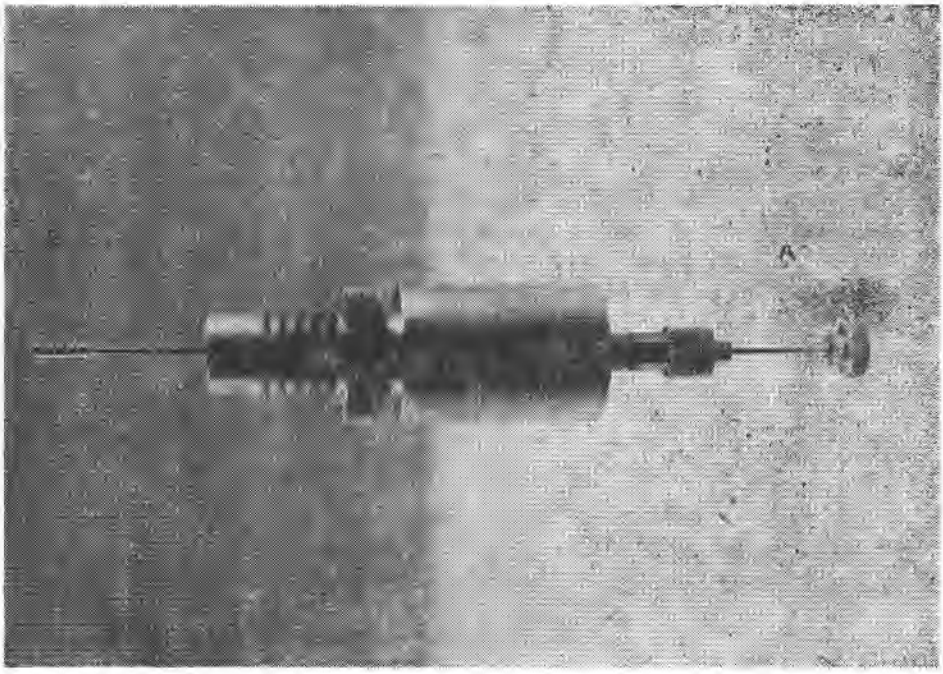

Fig. 1. The photograph of the apparatus used for the injection of the plant material

For the direct gas chromatography of the plant material a special apparatus, an injector, which is shown in Fig. 1, is used (8).

The main part of the injector consist of a stainless steel rod, A, with a male screw at one end where different carriers or holders for solid samples can be fitted. The rod passes through a gas - tight septum and can easily be moved into and out of the metal tube. Samples are deposited into the holder, B, and then the metal tube is screwed on the injection port inlet of the gas chromatograph. A sample can be injected by pushing the stainless stell rod forward so that the sample is brought into the flash heater. Carrier gas which enters through, carries the evaporated components into the column.

In this research a basket was used as a holder (Fig. 1. B). This basket is consist of a stainless steel tube 2 cm long with inner diameter of 2 mm and it has several holes. One end is closed and the other end is screwed at the end of the stainless steel rod, A. 8 - 10 mg of the plant material (five or six leaves and two - three flowers) were placed in the basket and then the metal tube was screwed on to the injection port. The rod A was pushed forward and was kept there for 30 seconds. During this period the carrier gas sweeps the evaporated components through the holes of the basket into the column. Then the rod was pulled back. This apparatus was left on the chromatograph till the end of the analysis.

Identification of the compounds was performed by gas chromatography of authentical samples of the monoterpene hydrocarbons and oxygenated compounds (*).

RESULTS and DISCUSSIONS

Monoterpene hydrocarbon fraction and the oxygenated compounds of the volatile oil isolated by steam distillation and fractionated on silica gel column is found to be in this portion :

The volatile oil contains 13 per cent monoterpene hydrocarbons and 87 per cent oxygenated compounds.

(*) I would like to express my gratitude to Prof. Dr. A. Baerheim SVENDSEN, who gave me the chance to work in his laboratory, in Leiden (Niederland).

Eleven (11) monoterpene hydrocarbons and 1 - 8 cineol were identified by gas chromatographic analysis on Carbowax 1540 (System I) and on β, β - oxydiproprionitrile (System II). These are α - pinene, camphene, β - pinene, sabinene, Δ_3 carene, α - phellandrene, α - terpinene, limonene, β - phellandrene, γ - terpinene and p - cymene. The main compounds are α - pinene, camphene, β - pinene, sabinene, α - phellandrene, limonene, p - cymene and 1 - 6 cineol

The gas chromatographic relative retention times of identified monoterpene hydrocarbons are given in Table 1.

Table 1. Relative retention times of monoterpene hydrocarbons of **Thymus sipyleus** volatile oil on Carbowax 1540, 70°C (Syst. I) and on β, β - oxydiproprionitrile, 35°C (Syst. II) columns.

	Terpenes	System I	System II
(1)	α - Pinene	4,7	3,5
(2)	Camphene	6,1	5,4
(3)	β - Pinene	7,6	7,0
(4)	Sabinene	8,3	9,9
(5)	Δ_3 Carene	9,6	8,9
(6)	α - Phellandrene	10,4	14,4
(7)	α - Terpinene	11,5	
(8)	Limonene	12,8	13,8
(9)	β - Phellandrene	13,8	16,4
(10)	γ - Terpinene	16,2	20,6
(11)	1 - 8 Cineol	18,0	28,1
(12)	p - Cymene	21,2	38,0

The volatile oil which was obtained by steam distillation was also chromatopraphed under the same conditions. By comparing the gas chromatograms it can easily be seen that the volatile oil gaves the identical picture.

The typical gas chromatograms of the monoterpene hydrocarbons and of isolated oil on Carbowax 1540 (Syst. I) and on β, β, oxydiproprionitrile (Syst. II) columns are shown in Fig. 2 and Fig. 3.

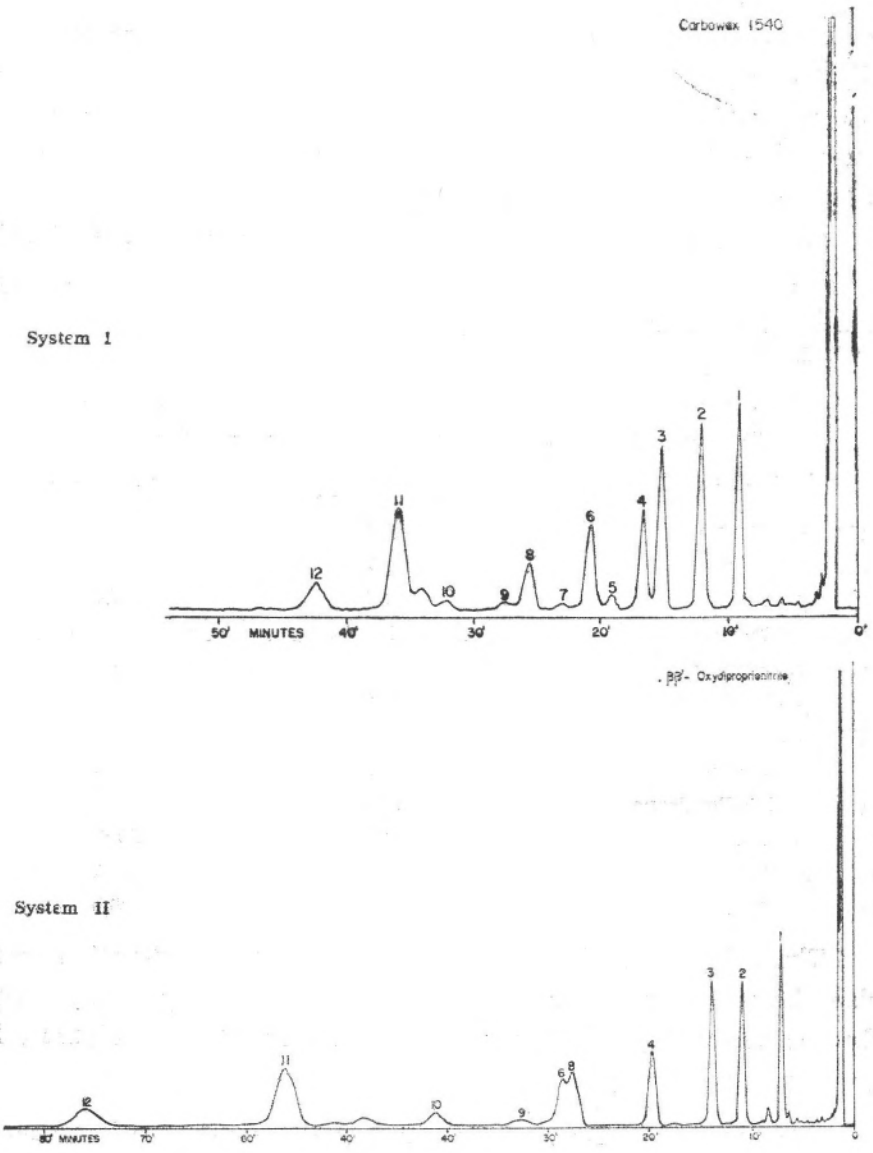

Fig. 2. Gas chromatograms of the monoterpene hydrocarbons on System I and on System II. (The experimental conditions as described under «Experimental»). For number refer to Table 1.

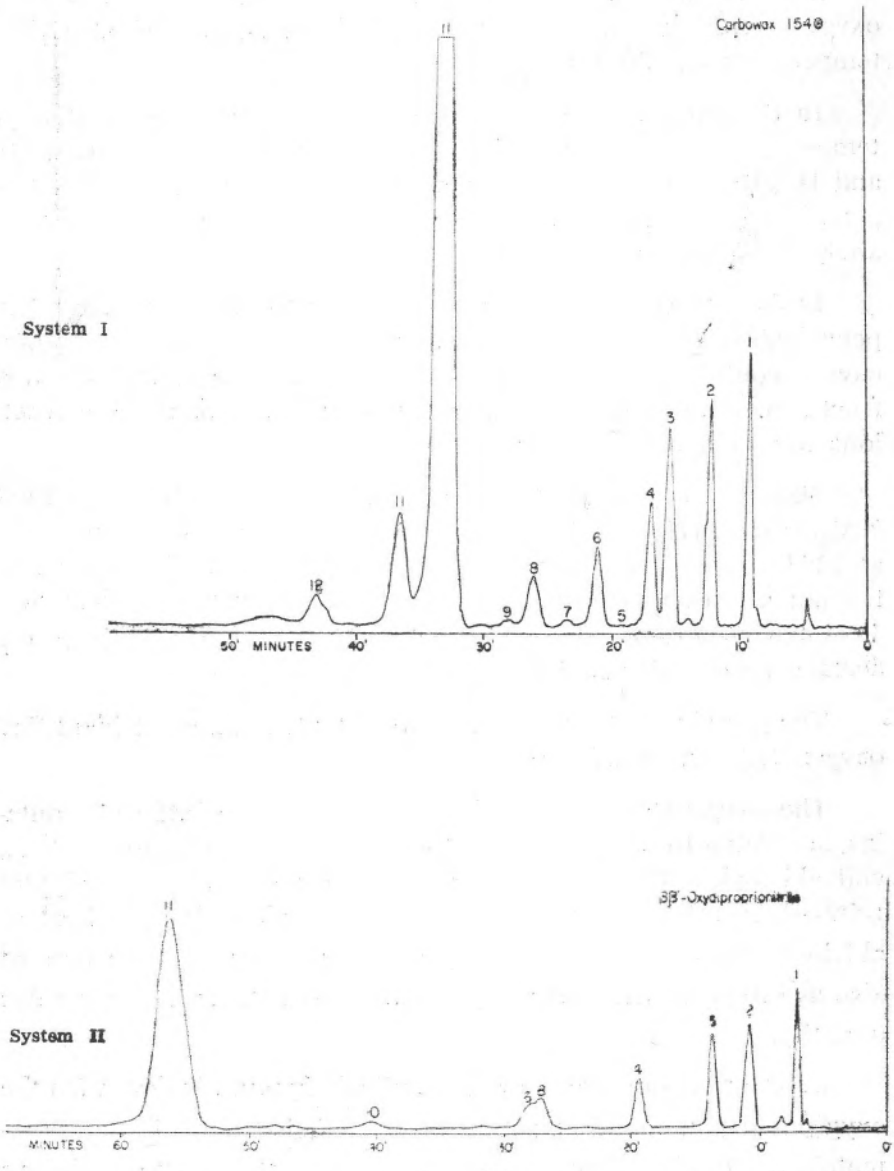

Fig. 3. Gas chromatograms of **Thymus sipyleus** oil on System I and **System II.** The experimental conditions as described under «Experimental». For number refer to Table 1.

After separation of the hydrocarbon fraction, higher boiling oxygen containing monoterpenes gas chromatographed at higher temperature on different columns.

In System I and II, stationary phases decomposing at high temperatures are present. Those stationary phases in System III and IV are stable at high temperatures. For this reason monoterpene hydrocarbons easily separated at low temperatures must be analyzed in System I and II.

In System III and IV it will be better to sweep the monoterpene hydrocarbons quickly and then to separate higher boiling oxygen containing monoterpenes. In this way, the volatile oil was fractionated through silica gel column first and then these fractions were analyzed in different systems.

Sixteen (16) oxygenated compounds were identified on PEG 20M (System III) at 140°C and also on Carbowax 1540 (System IV) at 125°C. These are eucalyptol (cineol), 6-methyl-5-heptenone, fenchone, thujone, citronellal, linalool, bornyl acetate, terpinene - 4 - ol, terpineol, borneol (and isoborneol), citral, citronellol, geranly acetate, nerol and geraniol.

The gas chromatographic relative retention times of identified oxygenated compounds are given in Table 2.

The oxygenated compounds fraction of the volatile oil contains, according to our gas chromatographic studies, alcohols, cetons, and aldehydes. The main components seemed to be eucalyptol (cineol), linalool, terpineol, citronellol as alcohols and citral as aldehyde. Two of the alcohols, geraniol and borneol were present also as esters, of which geranly acetate is seen to be in cosiderable quantity.

In order to compare the volatile oil of *Thymus sipyleus* with the oxygenated compounds fraction of the oil, isolated oil was chromatographed in the same circumstances on the same columns (System III and System IV). The gas chromatograms of each sample are shown in Fig. 4.

Table 2. Relative retention times of oxygenated compounds fraction of **Thymus sipyleus** volatile oil on PEG 20M, 140°C (Syst. III) and on Carbowax 1540, 125°C (Syst. IV) columns :

	Oxygenated compounds	System III	System IV
(101)	Eucalyptol	4.0	2.9
(102)	6 - methyl - 5 - heptenone	5.3	4.7
(103)	Fenchone	7.8	6.5
(104)	Thujone		7.4
(105)	Citronellal	8.5	8.2
(106)	Linalool	9.8	10.0
(107)	unknown	11.4	10.9
(108)	Bornyl acetate	13.1	12.7
(109)	Terpinene - 4 - ol	13.4	13.6
(110)	Terpineol	18.7	20.7
(111)	Borneol	19.4	18.6
(112)	Isoborneol		17.0
(113)	Citral	23.5	25.6
(114)	Citronellol		25.6
(115)	Geranyl acetate	22.5	26.9
(116)	Nerol	27.4	32.6
(117)	Geraniol	32.8	40.7

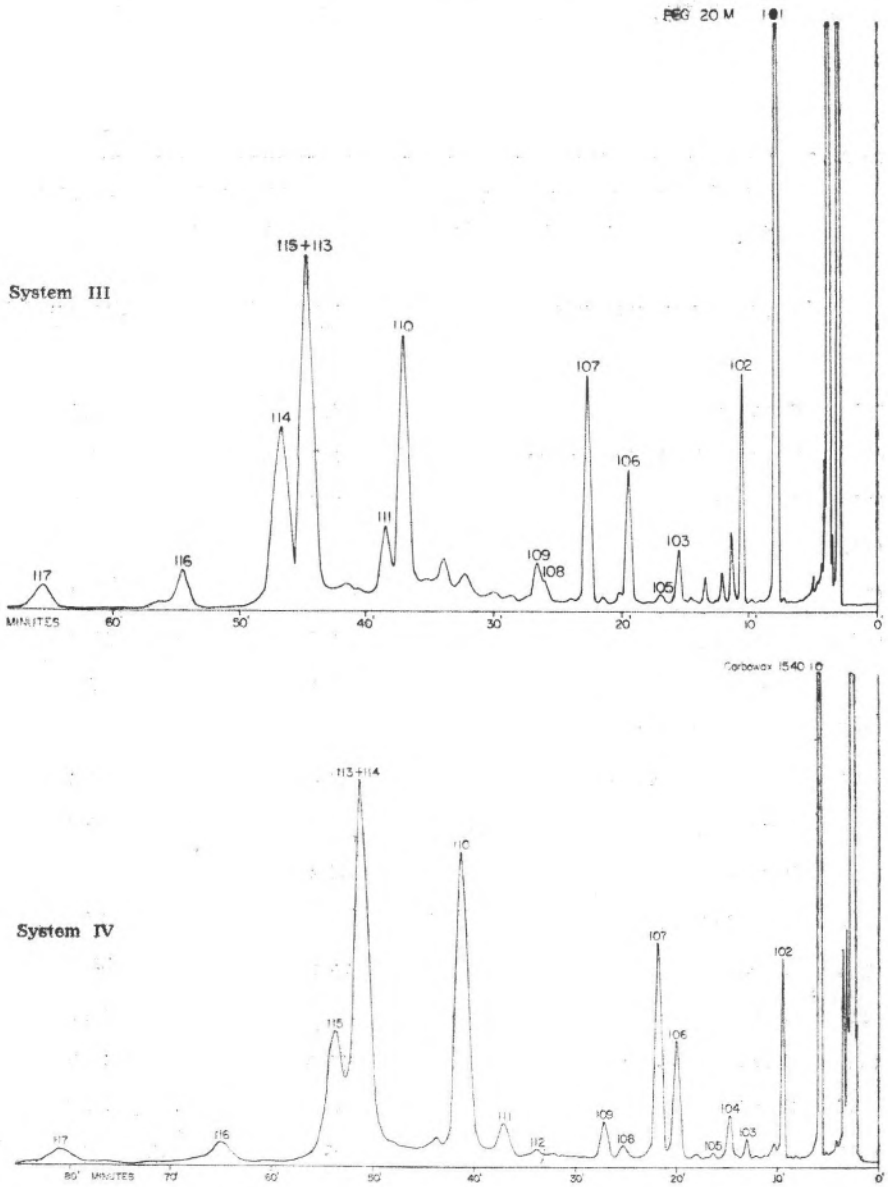

Fig. 4. Gas chromatograms of the oxygenated compounds on System III and System IV. (The experimental conditions as described under «Experimental»). For the number refer to Table 2.

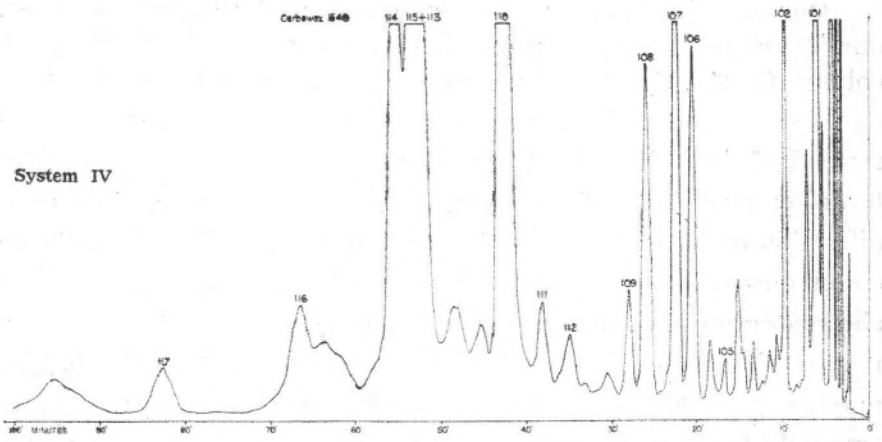

Fig. 5. Gas chromatogram of **Thymus sipyleus** oil on **System IV.** The experimental conditions as described under «Experimental»). For the numbers refer to Table 2.

The peak numbered as 107 was thought, according to our chromatograms and compared with pure substances, to be linalyl acetate. But, after saponification, no change has been observed in that peak. In fact, linalyl acetate is one of the esters which saponifies very easily. So, this compound should be something else. For the present we left it unknown. (Later studies will solve this)

The presence of thymol is not to be found in the *Thymus sipyleus* volatile oil.

Our gas chromatographic analysis showed that the greatest number of oxygenated compounds can be separated on PEG 20M column (Syst. III) and on Carbowax 1540 column (Syst. IV). But, citral, citronellol and geranyl acetate were giving mostly two peaks instead of three (Fig. 4.). Since, one of these compounds is an ester, after saponification we could get rid of it. Actually, after saponification only one peak was left, numbered as 114. This could be either citronellol or citral or citronellol + citral together. On the chromatograms which were taken after saponification, a very interesting change was seen (Fig. 5 and Fig. 6). The peak height, numbered as 102, increased. Citral decomposes and converts to 6methyl-5-heptenone easily, boiling with KOH (during the saponification procedure) (12). To check this change, pure citral was treated with KOH, in the same circumstances. The product was gas chromatographed, and on the chromatogram nothing was left at the citral point, the peak which belongs to citral was disappeared, but there was a higher peak at number 102, as methyl heptenone.

After getting such a result it became very obvious that the peaks 113, 114 and 115 belonged to geranly acetate, citral and citronellol. Geranly acetate saponified and so geraniol peak became higher, citral converted to 6-methyl-5-heptenone and the peak which left behind after saponification had to be citronellol.

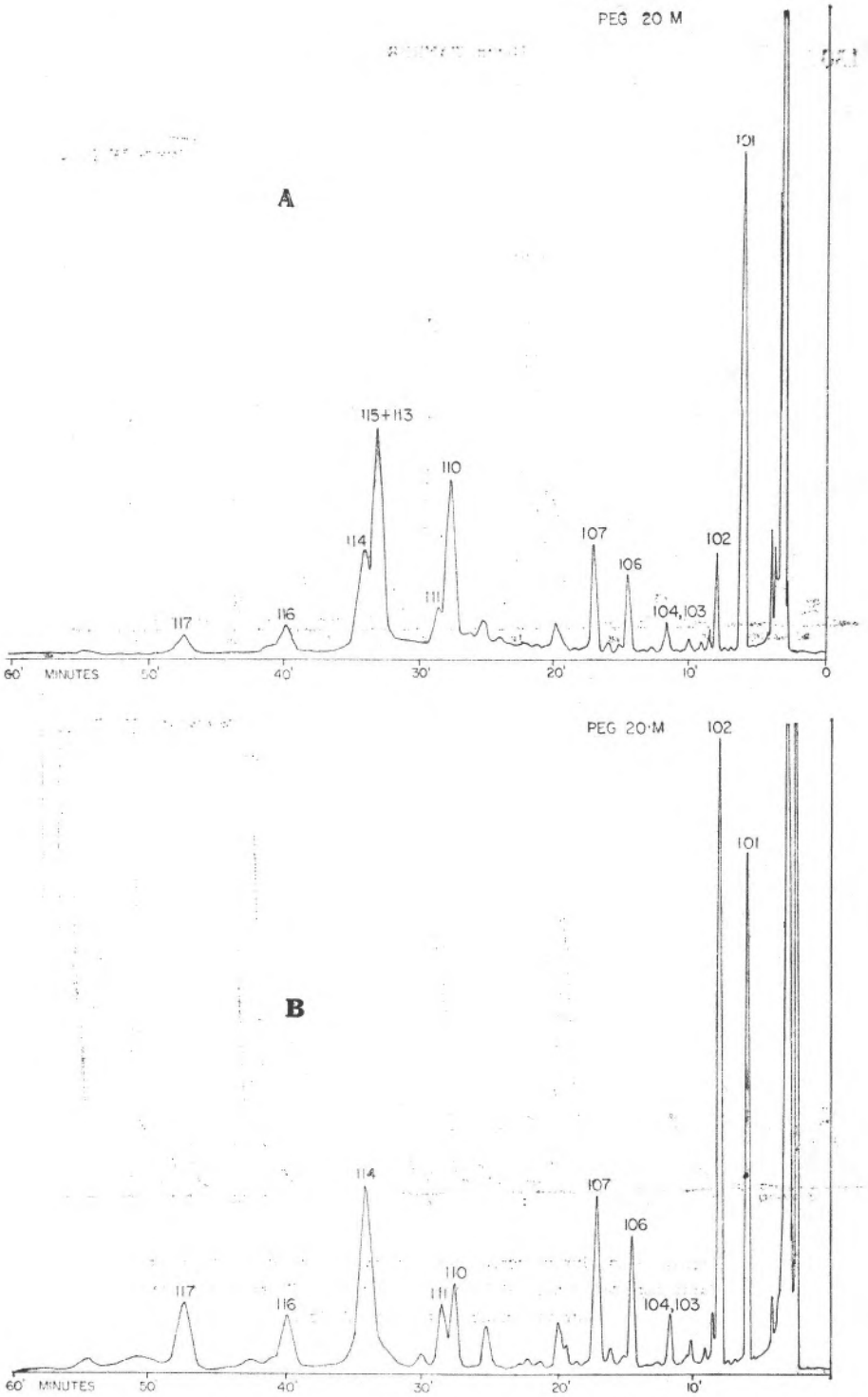

Fig. 6. Gas chromatograms of the oxygenated compounds on PEG 20 M (Syst. III) before (A) and after (B) sapenification. For numbers refer to Table 2.

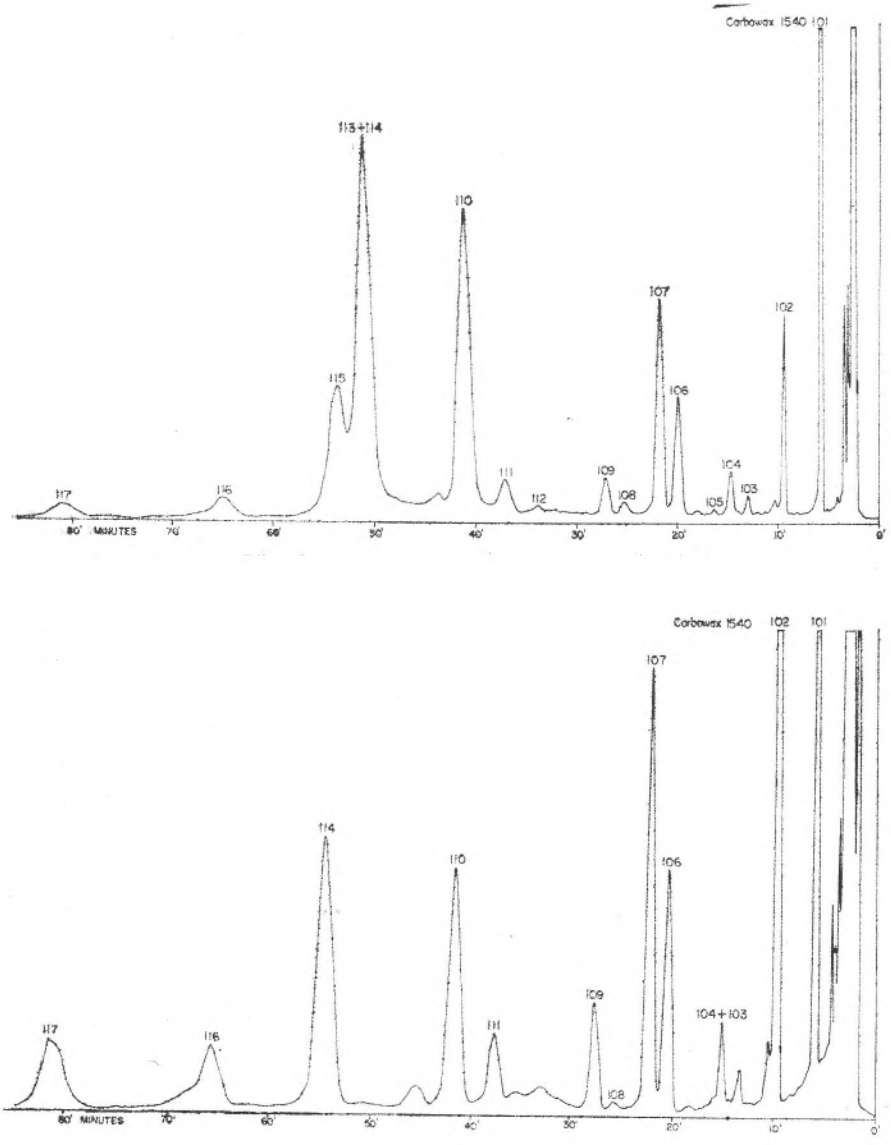

Fig. 7. Gas chromatograms of the oxygenated compounds on Carbowax 1540 (Syst. IV) before (A) and after (B) sapenification. For the numbers refer to Table 2.

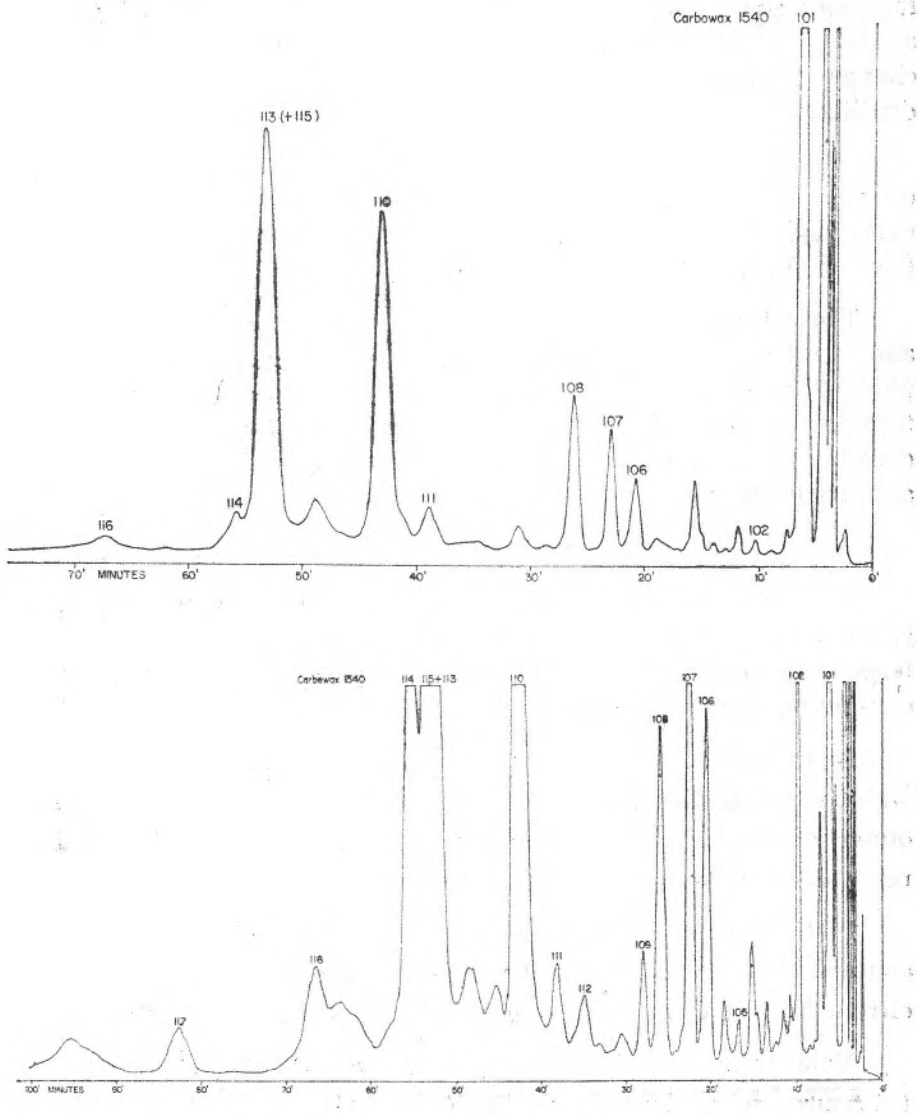

Fig. 8. Gas chromatograms of the plant material (A) and volatile oil (B) on Carbowax column (Syst. IV) at 125°C. For numbers refer to Table 2.

The odour of plants is due to the volatile substances they contain. But sometimes a volatile oil may not have the same odour as the plant from which it is isolated. This fact shows that some changes take place in the components of the volatile oil during the distillation.

In order to make a comparison the plant material, the volatile oil and the oxygenated compounds fraction of the oil were chromatographed under the same conditions on Carbowax 1540 column (System IV), at 125°C (Fig. 7).

These three chromatograms show that, eucalyptol, terpineol and citral are present in considerably great amount in the living plant. The main components of the oil are eucalyptol, linalool, terpineol, citronellol, geranly acetate and citral. Some of the citral decomposes during the steam distillation and so methyl heptenone appears in the oil.

SUMMARY

The lemon-like smelling volatile oil of *Thymus sipyleus* Boiss. growing in Beynam Forest (Ankara), contains 13 per cent monoterpene hydrocarbon fraction and 87 per cent higher boiling oxygen containing monoterpene fraction.

In this oil eleven (11) monoterpene hydrocarbons and eucalyptol were identified in the hydrocarbon fraction. The main compounds of this fraction were α-pinene, camphene, β-pinene, sabinene, α - phellandrene and limonene.

In the second fraction of the oil seventeen (17) oxygenated compounds were identified of which eucalyptol, linalool, terpineol, citronellol and citral were found to be the main components.

Volatile oil contains 21 % of citral and 13 % of eucalyptol (measured with planimeter).

The presence of esters, such as geranly acetate and bornyl acetate was verified by saponification of the oxygenated compounds fraction.

Four systems were used for the gas chromatographis analysis. Two of them had the stationary phases suitable for to use at lower temperatures (System I and II) and the other two had stationary phases which was stable at higher temperatures (System III and IV).

Several chromatograms which belong to volatile oil, monoterpene hydrocarbon fraction, oxygenated compounds fraction and direct plant material were compared each other.

By introducing the plant material directly to the gas chromatograph it was found that in living plant citral, eucalyptol and terpineol were present in considerably great amount. The odour of the plant and volatile oil confirmed this.

The presence of thymol is not to be found in *Thymus sipyleus* volatile oil.

ÖZET

Türkiye'de uçucu yağ endüstrisi hemen hemen sadece Isparta-Burdur bölgesinde ve gül yağı elde edilişi bakımından gelişmiş bulunmaktadır. Gülyağı ve gül konkretine ilâveten Alanya'da elde edilen yasemin konkreti de yurt dışına satılmaktadır.

Diğer taraftan Türkiye yılda 170 ton civarında uçucu yağ ithal etmektedir. Türkiye'nin ithal ettiği uçucu yağların başında, yılda 101 ton ile sitronella gelmektedir (*). *Cymbopogon* türlerinden elde edilen ve limon kokulu bir uçucu yağ olan sitronella, daha ucuz olduğu için limon esansı yerine kullanılmaktadır. *Cymbopogon* memleketimizde yetişmediğine göre aynı maksatla kullanılacak başka bir bitkinin aranması ve çoğaltılması ve uçucu yağının elde edilmesi, Türkiye'deki uçucu yağ endüstrisine katkıda bulunacak bir husustur.

Bu çalışma Beynam Ormanı'nda (Ankara) yetişen ve limon kokulu bir bitki olan *Thymus sipyleus* Boiss.'ın bu açıdan değerlendirilmesi amacıyla yapılmış ve bu türden elde edilen uçucu yağın bileşimi gaz kromatografisi yardımıyle açıklanmıştır.

(*) Aylık Dış Ticaret İstatistikleri 1972.

Thymus sipyleus'un su buharı distilasyonuyla elde edilen uçucu yağı % 13 monoterpenik hidrokarbon fraksiyonu ve % 87 yüksek temperatürde kaynıyan ve oksijen taşıyan monoterpen fraksiyonu ihtiva eder.

Bu uçucu yağda monoterpenik 11 hidrokarbon ve oksijenli monoterpenlerden 17 madde teşhis edilmiştir. Bunların başlıcaları α - pinen, kamfen, β - pinen, sabinen, α - fellandren, limonen; ökaliptol, linalol, terpineol, sitronellol ve sitraldır.

Uçucu yağda % 21 sitral ve % 13 ökaliptol bulunmuştur (planimetre ile yapılan tayine göre).

Esterlerin varlığı, oksijenli monoterpenlerin bulunduğu fraksiyonun sabunlaştırılmasıyle saptanmıştır.

Gaz kromatografisi ile yapılan analizlerde, ikisi alçak temperatürdeki çalışmalar için uygun olan (Sistem I ve II), diğer ikisi ise yüksek ısıya dayanıklı stasyoner fazlar ihtiva eden (Sistem III ve IV) olmak üzere 4 sistemden faydalanılmıştır.

Uçucu yağa, monoterpenik hidrokarbür fraksiyonuna ve oksijenli bileşikler fraksiyonuna ait olan ve ayrıca doğrudan doğruya bitki enjekte edilmek suretiyle elde edilen muhtelif kromatogramlar birbiriyle mukayese edilmiştir.

Doğrudan doğruya bitki enjekte etmek suretiyle elde edilen kromatogramlarda sitral, ökaliptol ve terpineol'ün oldukça fazla miktarda bulunduğu anlaşılmıştır. Bitkinin ve uçucu yağının karakteristik kokusu bu sonucu doğrulamaktadır.

Bu türde timol mevcut değildir.

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