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COMPONENT COMPOSITION OF THE ESSENTIAL OIL OF *Thymus dimorphus*

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The composition of the essential oil (EO) of Thymus dimorphus *Klok. et Shost. has been studied with the aid of chromato-mass spectroscopy for the first time, and 80 components of this oil have been identified. The EO of this thyme is represented mainly by terpenes and their alcohols and by sesquiterpenes and their alcohols. The total proportions of terpene and sesquiterpene derivatives are approximately the same. It has ben shown that with such a composition of the EO the widely employed polar columns of Carbowax 20M are not very effective.*

With the aim of expanding the raw materials base for the pharmaceutical industry, a search is being made for new promising essential-oil plants, and, in particular, *Thymus dimorphus* Klok. et Shost. is being investigated. This is a hybrid species widely distributed in the steppe regions of Ukraine, its origin being connected with natural hybridization between populations of *T. marschallianus* Willd. and *T. calcareus* Klok. et Shost. *T. dimorphus* is highly polymorphic species possessing the characteristic of both maternal species, but, in contrast to *T. marschallianus*, it has long procumbent rooting shoots and petiolate leaves, prefers dryer habitats, and is undemanding as to soil quality. In contrast to the endemic *T. calcareus*, which is restricted to chalky outcrops, it is found on all substrates and has a lacerate and noncapitate inflorescence [1].

The main component responsible for the pharmaceutical use of thyme is the essential oil (EO). *T. serpyllum* L., *T. vulgaris*, L.and *T. marschallianus* are used to obtain essential oils [2]. There is no information in the literature on the composition of the EO of *T. dimorphus*.

The composition of an EO can be determined by the preparative chromatographic isolation of the components, followed by their structural identification using various physicochemical methods (UV, IR, ^1H and ^{13}C NMR spectroscopy, etc.) and by combined methods (chromato-mass spectrometry — CMS, GLC—IR, HPLC—MS, etc.). The first route provides the possibility of a more concrete structural assignment but is laborious and does not permit the isolation of minor components (substances present at a concentration of $\geq 0.1\%$). The second route is more promising: it is economical with time and the chromatographic peaks are, as a rule identified by two independent methods — chromatographic (retention indices) and spectrometric.

The CMS combined method predominates in the elucidation of the component composition of an EO [3, 4], which is connected with its high sensitivity, selectivity (down to 1 pg/g), and specificity [5], which are achievable by the use of various types of capillary columns with a large number of stationary phases and various experimental conditions [6—9].

By working in the MID (multi-ion detection [8]) regime it is possible not only to increase the sensitivity of the methods by 2—3 orders of magnitude but also to improve sharply the efficiency of chromatographic separation. For this purpose 2-3 ions characteristic for each group of terpenoids are selected, and mass chromatograms are recorded for the selected ion currents [9]. For example, for the class of terpenes [7] these are the ions $[M^+]$ (the molecular ions), $[M - CH_3]^+$, and $[(M - CH_3) - C_2H_4]^+$ with mass numbers (*m/z*) of 136, 121, and 93; the class of terpene alcohols is represented by the ions $[M - H_2O]^+$, $[(M - H_2O) - CH_3]^+$, and $[C_4H_7O]^+$, with *m/z* 138, 123, and 71 [9]; and the class of sesquiterpene hydrocarbons is determined by the ions $[M]^+$, $[M - C_3H_7]^+$, and $[(M - C_3H_7) - C_2H_4]^+$, with *m/z* 204, 161, and 133 [9], and so on. The synchronous appearance of the given three (or two—four) peaks in the mass chromatogram, with identical times of emergence from the chromatographic column, gives a practically unambiguous assignment to a concrete class of terpenoids.

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TABLE 1. Component Composition of the EO of *T. dimorphus* (polar column)

Component	Retention time	KI	Probability of the identification, %	Content, %	M^+
1. Pentanol	1.77			0.1	
2. α-Pinene	2.179	1036	94	4.0	136
3.Sabinene	2.179	1130	90	2.6	136
4.β-Myrcene	2.451	1156	90	6.0	136
5.Bornylene	2.590	-	84	5.3	136
6.1,8-Cineole	2.758	1218	94	9.8	154
7.γ-Terpinene	2.897	1251	91	12.67	136
8. *para*-Cymene	3.073	1275	91	7.0	134
9. Hept-1-en-3-ol	6.458	1275	91	7.0	134
10. Camphor	7.22	1534	95	3.6	154
11. β-Bourbonene	8.735	1530	82	0.7	204
12. Terpinen-4-ol	10.359	1637	90	1.1	154
13. *trans*-Caryophyllene	10.580	1617	84	1.0	204
14.β-Bisabolene	10.664	1645	87	3.9	204
15. Sesquiterpene	11.890	-	-	1.7	204
16. Bornel	13.202	1689	90	1.6	154
17. Linalolyl propionate	13.636	1696	90	0.3	210
18. α-Cubebene	14.349	1658	87	5.4	204
19. Sesquiterpene	14.789	-	-	2.8	204
20. Sesquiterpene	15.700	2000	89	2.8	204
21. β-Himachalene	15.863	1718	88	0.4	204
22. *trans*-Bergamenthol-2,12-dien-14-ol	16.811	-	87	0.4	220
23. Caryophyllene oxide	21.110	1700	86	1.0	220
24. Sesquiterpene	24.767	-	-	1.0	204
25. Nerolidol	25.880	1961	90	1.2	222
26. Spathulenol	26.886	1961	90	1.2	222
27. Carvacrol	28.372	2159	92	13.1	150
28. Sesquiterpene	28.760	-	-	10.2	204
29. Thymol	29.479	-	93	5.0	150
30. Sesquiterpene	30.132	-	-	2.1	204
31. Sesquiterpene	32.462	-	-	3.0	204

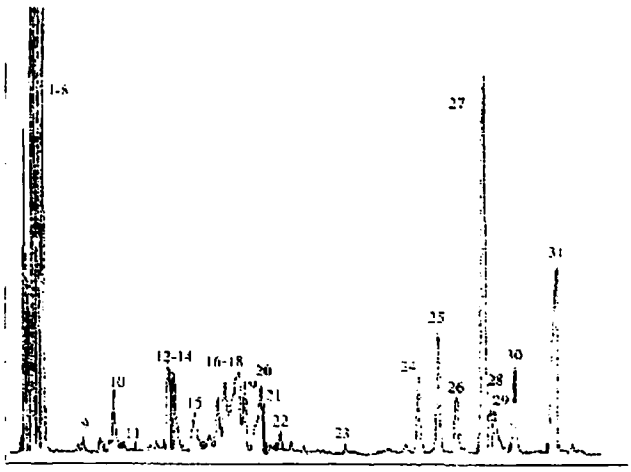

Fig. 1. Chromatogram for the full ion current of the essential oil of *T. dimorphus* obtained on a polar column of Carbowax-20M (axis of abscissas — retention time, min; axis of ordinates — full ion current).

TABLE 2. Component Composition of the EO of *T. dimorphus* (nonpolar column)

Component	Retention time	Kovats index/literature index	Probability of the identification, %	Content, %	M^+, amu
1. Benzene	1.78	529/-	90		
2. Methyl caproate	2.452	755/-	92	0.1/0.7	
3.β-Phellandrene	5.081	914/-	93	0.1/2.2	138
4. α-Thujene	5.40	925/938	84	0.2/2.5	136
5. α-Pinene	5.627	933/942	94	2.0/28.9	136
6. Camphene	5.97	943/-	94	0.8/12.3	136
7. Sabinene	6.85	943/976	91	1.6/24.0	136
8. β-Myrcene	7.899	989/984	90	8.5/127	136
9. Monoterpene	7.959	990/-	-	1.3/18.9	136
10. 1-Phellandrene	8.073	992/938	92	0.2/2.7	136
11. *para*-Cymene (impurity 1,8-cineole)	9.03	1017/1020	90	6.5/97.5	136
12. Limonene	9.37	1026/1030	90	6.5/97.5	136
13. *(Z)*-β-Ocimene	9.484	1034/1025	90	0.3/4.4	136
14. *(E)*-β-Ocimene	10.03	1047/1038	90	2.2/32.8	136
15. γ-Terpinene	10.288	1054/1057	92	1.1/16.9	136
16. *trans*-Sabinene hydrate	10.402	1057/1060	90	0.1/0.9	136
17. Dimethylstyrene	11.140	1056/-	90	0.1/0.9	136
18. α-Terpinolene	11.341	1080/1070	96	0.2/3.2	136
19. *trans*-Ocimene	11.859	1092/-	90	0.2/2.5	136
20. Camphor	13.085	1126/1126	95	0.9/13.5	154
21. 3,4-Ethenyl-1,2-dimethylcyclohexa-1,4-diene	13.673	1129/-	-	0.3/0.1	150
22. Borneol	14.461	1163/1164	84	1.0/16.3	154
23. 4-Terpineol	15.177	1181/1175	94	1.0/14.5	154
24. α-Terpineol	15.725	1194/1185	86	0.5/7.4	154
25. Carveol	16.102	1203/-	86	0.01/0.2	150
26.Terpen-?-ol	16.250	1207/-	-	0.01/0.1	154
27. Thymol methyl ether	17.611	1244/-	90	0.1/1.3	164
28. Carvacrol	21.19	1336/-	90	11.7/1.8	150
29. Thymol	21.40	1342/-	95	1.1/16.2	150
30. α-Cubebene	23.37	1392/1381	96	0.1/3.5	204
31. α-Ylangene	24.288	1415/-	94	0.2/2.4	204
32. Sesquiterpene	24.433	1421/-	-	0.6/8.5	204
33. β-Bourbonene	24.787	1430/1406	87	1.0/14.4	204
34. β-Elemene	25.082	1438/1400	90	0.6/8.6	204
35. α-Gurjunene	25.78	1457/1400	93	0.2/3.4	204
36. *trans*-Caryophyllene	26.373	1472/1428	98	3.5/5.2	204
37. Epibicyclosesquiphellandrene	26.636	1478/-	93	0.5/7.5	204
38. β-Gurjunene	26.815	1483/1435	86	0.1/2.2	204
39. Alloaromadendrene	27.022	1488/1475	93	0.4/7.4	204
40. Sesquiteree	27.171	1492/-	-	0.4/5.2	204
41. Cycloisolongifolene	27.50	1500/-	83	1.5/23.2	204
42. β-Guaiene	27.98	1513/1482	91	2.5/37.3	204
43. Sesquiterpenol ester	28.13	1518/-	-	0.2/3.3	204
44. β-Farnesene	28.34	1523/1448	84	0.5/6.9	204
45. δ-Cadinene	29.01	1541/1524	90	4.9/72.8	204
46. γ-Cadinene	26.096	-/-	-	1.5/23.1	204
47. β-Cubebene	29.307	1530/1381	91	0.6/9.1	204
48. γ-Elemene	29.547	1556/1425	90	2.2/32.3	204
49. α-Muurolene	29.852	1564/1500	97	1.5/22.7	204
50. ?-Cadinene	29.872	1566/-	91	0.3/5.1	204
51. γ-Muurolene	30.334	1576/1524	98	3.1/45.8	204
52. ?-Cadinene	30.947	1592/-	94	4.4/65.1	204

TABLE 2. (continued)

Component	Retention time	Kovats index/literature index	Probability of the identification, %	Content, %	M^+, amu
53. α-Cubebene	31.07	1594/-	90	0.4/5.3	204
54. γ-Muurolene	31.31	1600/-	96	1.8/12.3	204
55. α-Humulene	31.55	1607/1467	84	1.0/14.5	204
56. Dehydroaromadendrene	31.939	1618/-	85	0.3/3.2	204
57. Palustrol	32.118	1633/-	80	0.2/2.9	220
58. Nerolidol	32.511	1633/1553	92	4.1/6.0	220
59. *p*-Menth-3-en-9-ol	32.94	1645	86	0.4/5.5	154
60. Sesquiterpenol	33.418	1657/-	-	0.5/7.1	220
61. Sesquiterpenol	33.718	1665/-	-	0.4/5.2	220
62. Torreyol	33.895	1669/-	83	0.3/4.1	220
63. Sesquiterpen-?-ol	34.37	1682/-	-	0.4/0.5	220
64. Sesquiterpen-?-ol	34.522	-/-	-		220
65. δ-Cadinene	34.882	1694/-	83	0.9/13.1	204
66. Terpen-?-ol	35.040	1699/-	-	2.0/29.6	220
67. Terpen-?-O-CO-R	35.284	1705/-	-	0.3/4.6	-
68. Sesquiterpen-?-ol	35.662	1714/-	-	2.4/35.9	220
69. α-Guaiene	35.893	1722/-	90	0.4/5.7	204
70. α-Costol	36.170	1729/-	90	0.2/2.8	220
71. Terpene-O-CO-R	36.250	1731/-	-	0.2/2.2	-
72. Sesquiterpen-?-ol	36.920	1749/-	-	2.9/41.7	220
73. Sesquiterpene(di-i-Pr)	37.130	-/-	-	0.5/6.2	-
74. 9,10-Dehydroisolongifolen-?-ol	37.570	1782	86	0.2/2.5	254
75. Terpen-?-ol	37.980	1776/-	-	0.2/2.2	238
76. Widdrene/Spathulenol	39.510	1816/-	86	0.03/-0.3/0.2	220
77. Sesquiterpen-?-ol	39.570	1818/-	-	0.1/0.7	220
78. Sesquiterpen-?-ol	40.470	1841/-	-	0.1/0.7	220
79. *cis*-α-Copaen-8-ol	40.700	1847/-	93	0.3/3.7	220
80. 9-Aristolen-1-ol	40.940	1835/-	93	0.2/2.1	220
81. Sesquiterpen-?-ol	42.040	1884/-	-	0.2/1.5	220

However, it is difficult to make a concrete structural identification within a class of terpenoids because of the closeness of their mass spectra. It is just for this reason that the employment of various systems of "library search" using computers has given no satisfactory results even with the use of a five-factor system of conditions [10]. A more reliable method of identification is a comparison of the identities of specific ion peaks characterizing a definite class of terpenoids [7]. In the first place, in this case the choice of ions is unlimited and may be made arbitrarily, and in the second place this criterion of identification is most highly screened from the influence of experimental factors. For example, by comparing the ion intensities J_{121}/J_{91} in the monoterpene series it is possible to decrease the number of substances that are candidates in the performance of structural identification (in practice by an order of magnitude). Thus, with the aid of a library search, 45 substances were selected as candidates for β-pinene with an approximation of 90%; the ratio of the intensities of these ions that was found decreased the number of candidates to 2—4 representatives [7, 11].

Thus, in an investigation of an EO by the CMS method it is possible: 1) to establish the presence of a given compound or group (class) of compounds in the mixture, and 2) to identify the components of the mixture by using the mass spectrum obtained as an averaged one on repeated scanning of the chromatographic peak, with deduction of the column background, from the ratio of the characteristic ions within a definite class of terpenoids and from the chromatographic Kovàts indices (KIs) [12] coinciding with the figures in a catalog [13] within limits of error of from 2 to 15 units.

It has been shown previously [3, 4, 7, 11] that the use of a column of the polar Carbowax-20 M permits the practically complete separation of the components in the EOs of various plants but is not very effective when an EO contains a large amount of compounds of the sesquiterpene series and alcoholic analogs of the terpenoids.

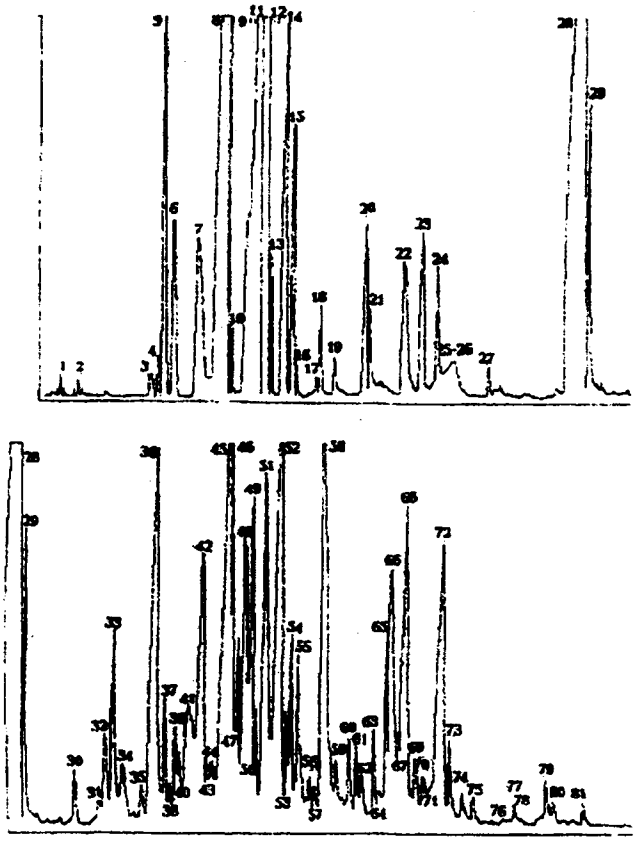

Fig. 2. Chromatogram for the full ion current of the essential oil of *T. dimorphus* obtained on a nonpolar column with the phase SE-30 (axis of abscissas — retention time, min; axis of ordinates — full ion current.

In the interpretation of the components of the EO obtained from *T. dimorphus* we used all the approaches described above (Fig. 1). The resolving capacity of the column used proved to be inadequate for the complete separation of the components of the EO, which, in its turn, excluded the possibility of the satisfactory use of KIs with an internal scale of normal hydrocarbons. As a result of this fact, the mean error of the determination of a KI amounted to about 10 units, instead of the 2—3 KI units in normal separation [11, 13]. This situation limited the possibility of identifying the components from literature KIs and in large degree was oriented to the order of elution of the components from the column and to mass-spectrometric results (finding of the M^+ peak, recording in the MID regime, and identification from the full mass spectrum with the inclusion of a library search [14]). Table 1 gives the identified components of the thyme EO. Representatives of the terpenes predominated, making up 30.5% of the total amount of terpenoids (predominantly having a cyclic nature). The amount of sesquiterpene hydrocarbons was approximately the same — 33.7%. There were representatives of terpene and sesquiterpene alcohols and also oxides of sesquiterpene hydrocarbons. An attempt was made to derivatize this EO with hexafluorobutyric anhydride [15] with the aim of obtaining the corresponding hexafluorobutyric esters in order to increase the resolving capacity of the Carbowax-20M column. In actual fact, this operation revealed a considerable number of new components in the region of elution of sesquiterpenes, but the absence from the literature of their mass spectra (M^+ was not recorded under electron-impact conditions) and of their KIs did not permit a reliable identification of the sesquiterpene alcohols. This operation only showed the unsatisfactoriness of the use of a polar column in a concrete case.

The predominance of representatives of the sesquiterpene hydrocarbons and alcohols, and also the rather poor separation on a column of the phase Carbowax-20M (Fig. 1) impelled us to make an additional investigation on a neutral column.

A chromatogram of the thyme EO recorded for the full ion current using a SE-30 column gave a different pattern of separation of the EO into its components (Table 2 and Fig. 2).

The main components characterizing the composition of the thyme EO were: α-pinene, β-myrcene, *para*-cymene, limonene, *trans*-β-ocimene, carvacrol, *trans*-caryophyllene, α-guaiene, terpenol, and sesquiterpene alcohols.

EXPERIMENTAL

The raw material for the investigation was gathered close to the village of Chapaevka, Vol'nyanskii region, Zaporozhskaya oblast in the period of mass flowering. The tips of leafy shoots with flowers were selected. The essential oil was isolated by steam distillation, its yield amounting to about 2%. Analysis of the component composition of the EO was made by the CMS method using capillary columns with different polarities. The components being eluted were identified by comparing their mass spectra with library information (library search method), comparing the ratios of the intensities of characteristic ions, and using Kovats retention indices according to an external scale of normal hydrocarbons. In doubtful cases of the structural assignment of an unknown component of the EM, a mass-fragmentogram was recorded of ions specific for classes of terpenoids (the MID method) and the samples were subjected to derivatization with hexafluorobutyric anhydride [15] in order to obtain mass spectra of esters of the terpene and sesquiterpene alcohols, since in an underivatized sample the M^+ peaks were absent, which made interpretation substantially more difficult.

We used a Hewlett-Packard (USA) model 5988A chromato-mass spectrometer. Capillary columns: 1) 25 m long, internal diameter 0.20 mm, thickness of the stationary phase, Carbowax-20M (HP-20M), 0.20 μm; 2) 30 m long; internal diameter 0.25 mm, thickness of the stationary phase, SE-30 (RSL-150), 0.20 μm. Separation conditions: initial temperature 60°C (held for 4 min), heating to 200°C at a rate of 3°C per min for column 1 and 2. After reaching a temperature of 200°C a 1-min pause followed by heating to 270°C at a rate of 10°C per min. Carrier gas helium, pressure at the inlet to the column 4 atm, split of the flow of carrier gas 1:20. Temperature of the chromatograph injector 250°C, temperature of the gas chromatograph—mass spectrometer interface 200°C. Time of recording a chromatogram 40—60 min.

The conditions for recording the mass spectra were the standard ones: electron-impact, 70 eV, temperature of the ion source 200°C, rate of scanning 8 scans/s from 39 to 450 amu. The mass spectra were recorded by means of a data-processing system, and by a special library search program a comparison was made of the mass spectrum obtained with a standard (Wiley library of 130,000 mass spectra). Variants with a degree of coincidence of $\lhd$90% were considered.

Quantitative evaluation of the amounts of the components of the EO was made by normalizing the areas of the chromatographic peaks on chromatograms obtained for the full ion current using components introduced for each class of terpenoids [14].

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