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GAS-LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY IN THE ANALYSIS OF ESSENTIAL OILS

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SUMMARY

An identification procedure for the components of essential oils, which frequently belong to the terpenoid series, is proposed based on gas-liquid chromatography-mass spectrometry. The basis of the method is a literature search with using the intensity ratio of the characteristic fragment ions in the mass spectrum and Kováts' indices. Essential oils of *Salvia garedznii* and *Ocimum gratissimum* were studied.

INTRODUCTION

The identification of volatile components of essential oils (EOs) by gas-liquid chromatography-mass spectrometry (GLC-MS) is carried out either by comparison with authentic samples, which is laborious, or on the basis of a literature search¹. In the latter case, the most difficult identifications are those of terpenoids which occur in the majority of EOs. Sasaki *et al.*² report that the correct identification of terpenoids is only possible in the case when MS, IR and NMR data are available. On the other hand, Mathews and Morrison^{3,4} have compared the various systems of literature search with particular reference to these compounds. Artemyev *et al.*⁵ and Adams *et al.*⁶ have developed algorithms for the identification of mono- and sesquiterpenoids. The results of the analysis of EOs are fairly good although further efforts in this direction are still required.

In this paper the components of the essentials oils of *Salvia garedznii* and *Ocimum gratissimum* are identified on the basis of a literature search using the intensity ratio of the characteristic fragment ions in the mass spectrum as well as Kováts retention indices.

EXPERIMENTAL

GLC-MS studies were made with a Varian MAT 311 A mass spectrometer. The energy of the ionizing electrons was 70 eV, temperature of the ionization chamber and of the separator 200°C, cathode emission current 300 mA and accelerating voltage 3 kV. Mass spectra and reconstructed chromatograms were obtained by automatic scanning in the range m/z 40-350 at 2-sec intervals.

GLC was performed with a Varian 3700 instrument having a glass SCOT column (20 m \times 0.2 mm I.D.); stationary phase, Carbowax 20M*⁷; initial temperature, 60°C; temperature programming rate 3°/min, final temperature 180°C followed by isothermal conditions; helium gas flow-rate 1.5 ml/min; temperature of the injection port and of the detector, 200°C. To improve the reproducibility of the quantitative and qualitative GLC data a specified amount of EO was introduced into the column prior to the experiment to saturate the active sorption sites⁸.

Kováts indices were determined without the use of internal standards. Each chromatographic column was first calibrated with the normal C₁₀–C₂₄ hydrocarbons. Chromatographic peaks were checked for homogeneity with the aid of the mass chromatograms for the characteristic fragment ions.

Quantitative analysis was made with a flame ionization detector. The relative concentrations of the components were found from calculations of the peak areas obtained with a TR-2215 integrator.

Terpenoids are very sensitive to experimental conditions. The spectra were therefore monitored at the minimum possible temperatures of the entire GLC–MS procedure. No systematic experimental error was found. The quality of literature mass spectra (1070) was tested by the program of Stenhagen *et al.*⁹.

RESULTS AND DISCUSSION

The effectiveness of a literature search is largely determined by the correct selection of the comparison criteria for mass spectra.

Earlier¹⁰ we evaluated the reproducibility of mass spectra in the GLC–MS mode on a single instrument and on a number of mass spectrometers. The mean error of the intensity measurements for all the fragment ions in the mass spectra was found 5% relative in the former case and 15% relative in the latter. Low-intensity ions are mainly responsible for the mean error. Therefore the introduction of a discrimination level of 5% relative seems reasonable.

The validity of narrowing mass spectra, as proposed by Talroze *et al.*¹¹, for terpenoid identification has been confirmed, independent of our studies, by Mathews and Morrison⁴ who demonstrated that this procedure does not affect the results of a literature search, no matter what comparison criteria are employed.

We put heavy reliance on an approach comprising the comparison of mass spectra by use of the intensity ratios of the characteristic fragment ions.

The following considerations have led us to do so: (1) this makes it possible to reduce significantly the number of fragment ions to be compared and (2) the criterion selected is mostly independent of the experimental conditions (a reproducibility of 5% relative).

The effectiveness of the approach adopted was tested on hydrocarbons, including terpenes with M⁺ having equal mass units up to 136 and with a major fragment ion at m/z 93. It is evident from Table I that a comparison relying on the intensity ratio of only three fragment ions at m/z 121, 92 and 91 reduces the number of possible candidates to one or two (four at most). If an unambiguous identification

* Carbowax 20M was taken as the base phase. This stationary phase is also recommended for use elsewhere⁷. In our opinion, the application of standardized columns to the analysis of EOs is a must.

TABLE I

COMPARISON OF THE MASS SPECTRA OF MONOTERPENE HYDROCARBONS BASED ON THE INTENSITY RATIOS OF FRAGMENT IONS

No.	Compound	I_{92}/I_{121}	I_{121}/I_{91}	No. of possible candidates	I_{92}/I_{93}	I_{80}/I_{93}
1	7-Isopropylidenebicyclo- [4,1,0]heptene	0.20	1.79	—	—	—
2	Spiro[4,5]decene-1	0.25	0.63	—	—	—
3	β -Phellandrene	0.31	0.32	—	—	—
4	β -Terpinene	0.31	0.41	—	—	—
5	4-(10)-Carene	0.35	0.14	—	—	—
6	Sabinene	0.36	0.24	—	—	—
7	4-Carene	0.43	1.66	—	—	—
8	7,7-Dimethyl-3-methylenebicyclo- [3,1,1]heptane	0.45	0.33	—	—	—
9	<i>Trans-m</i> -Mentha-4,8-diene	0.50	2.38	1	—	0.17
10	β -Pinene	0.53	0.71	2	0.75	0.09
11	Bornylene	0.54	0.62	2	—	—
12	Camphene	0.55	2.93	1	0.79	—
13	<i>m</i> -Mentha-1(7),8-diene	0.60	2.93	1	—	—
14	α -Fenchene	0.60	1.62	1	—	—
15	<i>cis-m</i> -Mentha-4,8-diene	0.65	0.92	2	0.99	—
16	Myrcene	0.67	0.46	—	—	—
17	α -Phellandrene	0.74	0.10	1	0.18	—
18	3-Carene	0.84	0.67	1	0.33	—
19	α -Thujene	0.87	0.11	1	0.08	—
20	γ -Terpinene	0.88	1.09	—	—	—
21	<i>trans-2</i> -Carene	0.97	0.50	2	1.94	0.17
22	β -Ocimene-x	1.00	0.58	4	0.03	0.70
23	β -Ocimene-y	1.00	0.72	2	0.14	0.47
24	<i>cis-2</i> -Carene	1.01	0.69	2	1.46	0.15
25	Tricyclene	1.04	1.45	2	0.72	0.19
26	α -Pinene	1.11	0.43	2	2.58	0.08
27	β -Thujene	1.17	1.52	1	0.50	0.30
28	Cyclofenchene	1.35	1.13	1	1.20	—
29	3,7,7-Trimethylbicyclo- [3,1,1]heptene-2	1.42	0.33	1	4.30	—
30	<i>m</i> -Mentha-1(6)-8-diene	3.80	0.88	—	—	—

is not achieved, use is made of additional criteria (intensity ratios of fragment ions characteristic of a given compound). For example, the intensity ratio I_{92}/I_{121} eliminates the uncertainty for tricyclene, β -thujene and cyclofenchene (0.72, 0.50 and 1.20, respectively). With this ratio it is possible to discriminate even between the structurally similar *cis*- and *trans-2*-carenes.

Analogous data were also obtained for other representatives of terpenoids. For instance, for sesquiterpene hydrocarbons such ions will be at m/z 204, 161, 119 and 105. An additional criterion is exemplified by a fragment ion at m/z 134 for δ -cadinene.

Sometimes the identification of EO components from the mass spectral data is difficult, especially in the case of stereoisomers. We compared the mass spectra of

TABLE II
MASS SPECTRAL DATA FOR MENTHANES

<i>Menthane</i>	Relative intensities of fragment ions, I_{max}/I_i				
	140	97	96	81	55
<i>cis-o-</i>	2.1	70.5	12.3	12.4	100.0
<i>trans-o-</i>	5.1	54.1	16.5	13.4	100.0
<i>cis-p-</i>	23.6	88.4	61.1	21.8	100.0
<i>trans-p-</i>	27.5	32.7	59.5	20.8	100.0
<i>cis-m-</i>	25.7	100.0	96.0	17.6	94.2
<i>trans-m-</i>	24.8	96.5	56.1	20.9	100.0

cis- and *trans-*isomers of *p-*, *m-* and *o-*menthanes (Table II). The effect of steric factors was found to be important only for the *o-*menthanes (the "ortho-effect"). The intensity ratio of the fragment ions, I_{97}/I_{140} , enables one to distinguish the *o-*menthanes (33.6 and 10.7) from their *p-* (3.7 and 3.5) and *m-* analogues (3.8 for the two isomers), and to establish their *cis-* and *trans-*isomerism, except in the case of the *m-*isomers.

However, the GLC-MS method enables these difficulties to be circumvented by the use of chromatographic data which are likely to be significantly different for compounds having similar mass spectra. Kováts' indices taken as a chromatographic characteristic are reproducible to ± 4 units, whereas such indices for most of the stereoisomeric pairs of mono- and sesquiterpenoids differ by 15-50 units. When account was taken of the results of interlaboratory studies into the reproducibility of Kováts' indices (at a mean relative error of 13 units), the range of the indices was found to be 20 units. This range permits the number of mass spectra to be reduced to 6-8.

Thus the following routine was adopted for the identification of components in EOs. The individual constituents are identified by successive use of chromatographic data, intensity ratios of the fragment ions typical of the compounds under study and additional criteria for given representatives. The identification is regarded as successful if the reproducibilities of the fragment ion intensities and Kováts' indices are within the experimental errors. This criterion permits a reliable analysis of EO to be made during a reasonably short time even without computers.

Verification of the identification method

The identification method proposed has been verified on known EOs whose components have been determined previously^{10,12}.

The described GLC-MS method was useful in studying the novel EOs possessing good organoleptic properties, *viz.*, those of *Lophanthus anisatus* (*Agastache foeniculum*)¹³, *Nepeta cataria* var. *Citriodora* Ball.¹⁴ and *Cymbopogon citratus* D.C.¹⁵.

The essential oil of Salvia garedznii N.A.Fr. *Salvia garedznii* N.A.Fr. is a xerophyte, endemic to the arid Caucasian zone. EO (up to 0.24%) is contained in the entire green stock of the plant. (The EOs were isolated from whole plants by steam distillation.) The composition of the EO of this species had not hitherto been studied.

TABLE III

QUALITATIVE AND QUANTITATIVE COMPOSITIONS OF THE ESSENTIAL OIL OF *Salvia garedznii* N.A.Fr.

No.	Compound	Molecular mass	Kováts' index	Content of EO (%)	Ref.
1	1,8-Cineol	154	1196	2.0*	16
2	3-Octanol	130	1362	2.3	17
3	Menthone	154	1432	0.3	18
4	Isomenthone	154	1451	1.7	18
5	Camphor	152	1455	25.7	19
6	β -Bourbonene	204	1490	1.0	20
7	Linalool	154	1548	4.0	16
8	Linalyl acetate	196	1563	1.0	16
9	Terpinen-4-ol	154	1557	2.0	16
10	Humulene	204	1607	0.7	21
11	Isobornyl propionate	210	1617	6.9	16
12	Terpinyl acetate	196	1622	1.7	22
13	Sesquiterpene hydrocarbon	202	1640	1.7	—
14	Borneol	154	1674	1.7	9
15	α -Terpineol	154	1679	3.0	9
16	Isobornyl butyrate	224	1704	10.0	9
17	Isobornyl valerate	238	1709	17.8	9
18	α -Curcumene	202	1762	1.3	23
19	Citronellol	156	1770	5.9	9
20	Nerol	154	1800	0.7	9
21	Geraniol	154	1848	2.3	9

* Percentage of the component in the monoterpene fraction equal to 30–90% of EO.

TABLE IV

QUALITATIVE AND QUANTITATIVE COMPOSITIONS OF THE SESQUITERPENE HYDROCARBONS OF *Ocimum gratissimum* L.

No.	Compound	Molecular mass	Kováts' index	Content (%)	Ref.
1	α -Cubebene	204	1442	0.7	21
2	α -Copaene	204	1460	7.2	17
3	β -Bourbonene	204	1490	3.6	17
4	β -Ylangene	204	1519	2.7	17
5	β -Caryophyllene	204	1572	39.5	9
6	β -Elemene	204	1582	3.9	9
7	Aromadendrene	204	1620	0.5	9
8	Humulene	204	1639	4.4	21
9	Alloaromadendrene	204	1673	0.2	9
10	Germacrene-D	204	1684	29.9	17
11	γ -Muurolene	204	1702	3.0	17
12	α -Muurolene	204	1707	0.5	17
13	γ -Cadinene	204	1740	0.5	17
14	δ -Cadinene	204	1745	3.0	17
15	Calamenene	202	1818	0.2	17

It does not contain (Table III) monoterpene hydrocarbons, and sesquiterpene hydrocarbons are represented by no more than three compounds; monoterpene ketones and alcohols are the major components. This EO differs from those of the other sages in that it contains a large amount of monoterpene alcohol ether.

The sesquiterpene fraction of the essential oil of Ocimum gratissimum L. The EO of *Ocimum gratissimum* L. is produced in the U.S.S.R. in large quantities for obtaining eugenol and isoeugenol. This oil also contains sesquiterpene hydrocarbons. Due to the fact that the sesquiterpene fraction contains a larger number of compounds than was found earlier²⁴, we have repeated a study of this EO.

The described method was instrumental in identifying thirteen sesquiterpenes (Table IV) of different structures. The mass spectra of the previously described²⁴ α - and β -santalenes contain a most typical fragment ion at m/z 94, as reported elsewhere⁹, therefore no unduly complicated problems in their identification are anticipated. However, these santalenes have not been found by us in the sesquiterpene hydrocarbons. Note also the higher content of β -caryophyllene in the EO (39.5% of the total amount of the sesquiterpene hydrocarbons) as compared with the value reported previously²⁴.

Attention is drawn to the fairly high content of germacrene-D (29.9%) in the sample studied, which was not found previously²⁵⁻²⁷. The presence of germacrene-D was confirmed by its separation in the form of a complex with AgNO_3 and identification by UV, IR and NMR spectroscopies.

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