CHROM. 6340

ANALYSIS OF SPIKE OIL BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

The analysis of spike oil was carried out by gas chromatography–mass spectrometry without any preliminary separations, and some of the major components of spike oil were identified. The minor components were assigned at least to definite classes of organic compounds.

INTRODUCTION

Spike oil is a mixture of monoterpenes, their oxygen derivatives and sesquiterpenes. The analysis of such a complicated mixture by analytical gas chromatography in combination with other methods of identification would not give satisfactory results without preliminary separation[1–6], *e.g.*, distillation or preparative liquid–solid or gas–liquid chromatography. These procedures are time-consuming and generally require large amounts of samples and sets of standards.

It is the main purpose of this work to show the scope and limitations of gas chromatography–mass spectrometry (GC–MS) for the qualitative analysis of complicated mixtures of natural compounds without the use of standard compounds. Thus, for the structural identification, we had to consider only the recorded spectra of a particular compound and to compare them with the published data. The main advantage of this method lies in the possibility of obtaining satisfactory results from a small amount of unknown material during one working day and without any stock of standard compounds.

EXPERIMENTAL

For the analysis of the spike oil we used a commercial instrument, an LKB 9000 gas chromatograph–mass spectrometer. The separation of the mixture was carried out in the chromatographic unit of the LKB 9000 instrument on a 50 m long × 0.023 cm I.D. capillary column, coated with Carbowax 20M. The temperature was increased linearly from 100° at the rate of 2°/min, and the injection port was main-

heated at 275°. The column outlet was connected via a carrier gas (helium) separator with the ion source of the mass spectrometer. The temperatures of the separator and the ion source were 260° and 275°, respectively.

The total ion current monitor served as a detector for recording of chromatograms; the energy of the ionizing electrons was 70 eV. When the mass spectrum of a compound is recorded, this value of the ionizing energy is automatically switched to a standard value of 70 eV.

The mass spectrum of each separated compound was taken, at intervals of less than 5 sec, where the maximum concentration of the given compound was attained in the ion source of the mass spectrometer, i.e., at the top of the chromatographic peak.

If a gas chromatographic peak represented more than one compound, a series of mass spectra were taken at various points on the chromatographic curve. From such a series of spectra, it was possible to suggest the structures of compounds that were not separated under our experimental conditions (e.g., Fig. 1, peak 18).

For some minor components of the mixture, in addition to the spectra taken at the top of chromatographic peak, the background spectra were also recorded and these spectra were subtracted from the former.

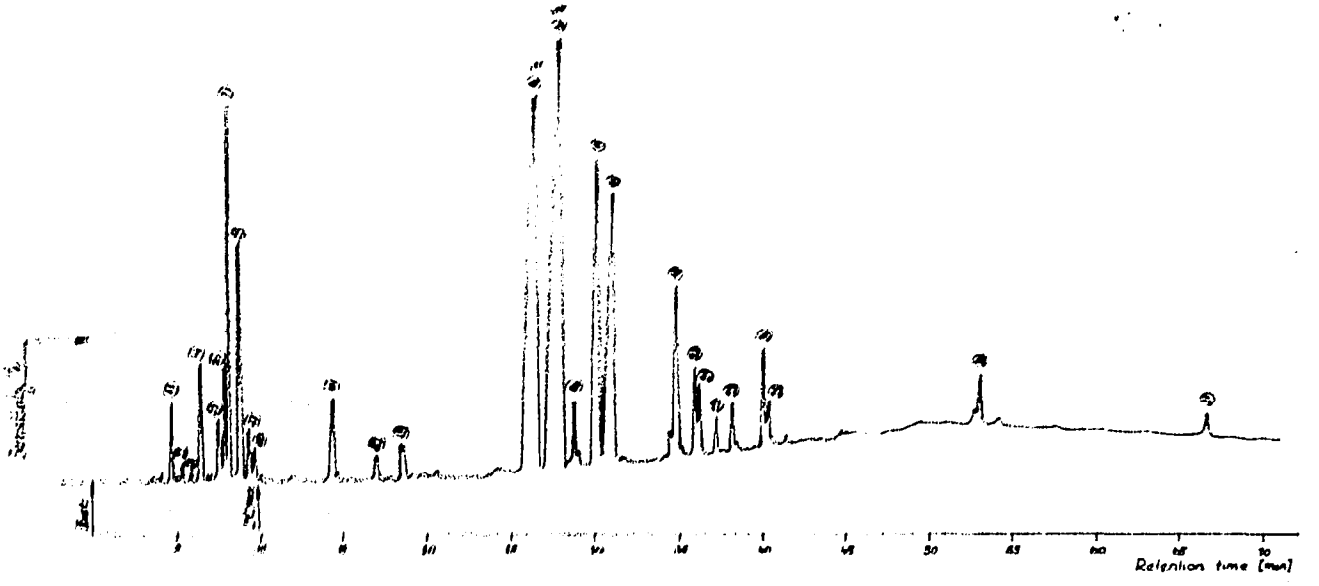

Fig. 1. Chromatogram of spike oil. Capillary column 50 m long coated with Carbowax 20M. Temperature of the column is increased from 100° to 200°. Peaks are identified in Table I.

RESULTS AND DISCUSSION

The chromatogram obtained from spike oil is shown in Fig. 1, and the results of the identification are summarized in Table I.

A reliable identification of the compounds under investigation by means of mass spectroscopy was possible only if the published spectra were available.

If the mass spectrum obtained was in complete agreement with the published spectrum, then only the name of the compound and the corresponding reference are given. If there was not absolute agreement with the published spectrum, a question

TABLE I

COMPOUNDS IDENTIFIED IN SPIKE OIL BY GC–MS

No.[a]	*Mol. weight*	*Compound*	*Reference*
1	136	α-Pinene	7
2	136	Camphene	8
3	136	β-Pinene	8
4	136	Myrcene	8
5	136	Limonene	7, 8
6	154	Cineol	9a
7	136	β-Ocimene-X	8
8	136	γ-Terpinene(?)[b]	8
9	144	*n*-Hexyl acetate	9b
10	134	*p*-Cymene	8
11	128	$CH_3COCH_2CH_2COC_2H_5$ or $CH_3COCH{=}CHCH(OH)$-CH_2CH_3	10a
12		Unidentified	11
13		Unidentified	10b
14	154	Linalool	12
15	196	Linalyl acetate	9c
16	204	Longifolene (?)[b]	9d
17	154	Terpinen-4-ol	13
18	204	β-Caryophyllene	14
	154	and lavandulol (?)[b]	13
19	204	β-Farnesene	15
20	154	α-Terpineol	13
21	154	Borneol	13
22	204	Unidentified sesquiterpene	16a
23	154	Nerol	13
24	196	Neryl acetate	9c
25	204	γ-Cadinene	16b
26	204	Unidentified sesquiterpene	
27	204	Unidentified sesquiterpene	

[a] Each compound number is identical with the number of its corresponding chromatographic peak in Fig. 1.

[b] The question mark (?) indicates that the spectrum has not been published or the identification is discussed in the text.

mark (?) is used, but this means that the difference between the obtained and published spectra is less significant than the difference between the mass spectrum obtained and the spectra of any other compounds that can be taken into consideration. These questionable spectra are further discussed.

A compound is considered to be "unidentified" if more than one structure can be proposed from the mass spectrum obtained.

In the spectrum of the chromatographic peak 8 there were differences in the abundances of some ions compared with the published spectrum of γ-terpinene[8]. Nevertheless, this discrepancy in the spectra is less than the difference between the available mass spectra of two monoterpenes of different structures.

The ions of *m/e* 57, 55, 43, 41, 85, etc. (characteristic of the fragmentation of a hydrocarbon skeleton), together with the ion of *m/e* 31, are dominant in the spectrum of chromatographic peak 13. These fragments indicate presence of an –X–CH_2–OX– group, where X = H or C, as well as a hydrocarbon chain in the structure of the molecule[10b].

Peak 16 has been identified as a sesquiterpene with a molecular weight of 204. Nevertheless, the ratio of the relative intensities of the most intensive ions of *m/e* 93, 91, 94 and 95 is almost equal to the ratio of the same ions in the spectrum of longifolene[9d] and differs strikingly from the spectra of all other sesquiterpenes.

Chromatographic peak 18 comprises two compounds. One of them has a spectrum which is in good agreement with the spectrum of β-caryophylene, which has been published[14]. This spectrum was obtained by the technique described in the EXPERIMENTAL section. The second component of chromatographic peak 18 has the values shown in Table II.

TABLE II

CHARACTERISTIC VALUES OF SECOND COMPONENT OF CHROMATOGRAPHIC PEAK 18

m/e	*Relative intensity (%)*
43	100
69	85
41	80
68	50
93	40
39	20
53	20
67	20
121	15
135	10

The spectrum of lavandulol[13] shows similar fragmentation. It is probable, therefore, that part of chromatographic peak 18 represents a molecular structure closely related to the structure of either lavandulol or its derivatives.

Compound 22 is a sesquiterpene (molecular weight 204) and its mass spectrum resembles the mass spectra of β-cubene[16a] and β-cedrene. It was not possible to give more details of the mass spectrum obtained from compound 22.

The high volatility of the liquid phase of the column at high temperatures (190°) made the identification of the structures of compounds 26 and 27 virtually impossible. Nevertheless, after subtraction of the background spectrum, it was possible to say that the both compounds 26 and 27 are probably sesquiterpenes with a molecular weight of 204.

CONCLUSION

The analysis of spike oil was carried out by using the LKB 9000 GC–MS instrument without preliminary separation treatments. The gas chromatograph of the instrument was used only for the separation of the mixture. The qualitative identification was based on the interpretation of the mass spectra recorded for each chromatographic peak. The relative retention times and Kováts indices were not used as further supports for the identifications.

The structures of the compounds that represent about 80–90% of the mixture were identified by the described technique. About 10% of the mixture is represented

by compounds that we are not able to identify from their recorded spectra. The remainder represents trace compounds for which the mass spectra were not recorded.

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