GAS CHROMATOGRAPHY OF OXYGEN-CONTAINING TERPENES

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It has been shown by several authors that gas chromatography is one of the more promising tools for the analysis of essential oils. Though several papers have been published on various oils no systematic investigation has been carried out on different classes of compounds.

In a previous paper[1] the behaviour of terpene hydrocarbons in gas chromatography has been described. The purpose of this work is to report the results obtained with more common oxygen-containing terpenes, which are usually found in citrus oils.The technique described should, however, be of value in the study of other terpenes from different sources.

The volatile oxygen-containing terpenes may be classified as alcohols, carbonyl compounds and esters. They have the following properties, which render chromatographic examination rather difficult: high sensitivity to heat and to the presence of foreign substances, which may cause their decomposition, and a high boiling point in quite a small range. Because of these features the working conditions should be chosen very carefully. Suitable working conditions have been established during this work and the retention volumes have been determined on different stationary phases, so that with these values the identification of the nature of an unknown terpene in an essential oil should also be possible.

EXPERIMENTAL

Apparatus

The chromatographic measurements were made with an apparatus provided with a gas density balance meter as detecting device.

Glass columns of 0.4–0.5 cm internal diameter were employed; they were connected by means of teflon tubing to obtain a total length of 3–5 m.

Solid supports

Various solid supports were tried. Substances such as sterchamol or firebrick gave unsatisfactory results; they do not behave as inert materials, since it was observed that several oxygen-containing terpenes (mainly alcohols and esters) are either strongly retained or decomposed. This is probably due to the presence of metal (iron, aluminum) oxides in these materials.

Celite is an excellent support provided inactivation of the material is carried out correctly. Embacel (May & Baker) has been found quite satisfactory.

Stationary liquids

It was found very convenient to perform the chromatographic separation on columns with quite different properties: one weakly polar, where the components are eluted according to their boiling point and the other strongly polar in order to achieve separations of compounds that can only be separated with difficulty. For the former D.C. 550 Silicone oil was used and for the latter Hyprose S.P. 80, trade name of the Dow Chemical Co. for octakis(2-hydroxypropyl)sucrose. The large number of hydroxyl groups makes this substance particularly suitable for the examination of polar compounds; it has been used up to 160° where its volatility is less than 0.1 %.

The stationary liquids were used in the ratio 20 % w/w. Silicone oil was dissolved in petroleum ether and Hyprose in methanol; after mixing, the solvent was eliminated by gentle heating. The use of low percentages of stationary phases is recommended, in order to obtain low retention volumes; this is one of the main requirements that should be fulfilled, since fairly long columns should be used in order to achieve separation of compounds that boil in a very close range.

A lower percentage of stationary liquid affects the shape of the elution peaks: a 20 % w/w concentration permits operation with a rather high gas flow-rate, which corresponds to a more convenient linear velocity. The best results were obtained with a gas flow-rate of 80–100 ml/min corresponding to a linear velocity of 15 cm/sec. These working conditions give a column efficiency of about 800 theoretical plates per meter of column.

Temperature

Particular attention should be paid to the column temperature; since most terpenes are very sensitive to heat, the columns should be kept at as low a temperature as possible. The compounds that were examined have a boiling range between 180° and 240°; they may be eluted under the conditions described in a fairly short time (60–100 min) by keeping the column temperature between 132°–156°.

These temperatures were obtained by means of boiling cellosolve ($t = 132°$) and cyclohexanol ($t = 156°$). The highest temperature that can be attained without causing decomposition depends on each substance; for instance the decomposition of linalyl acetate, one of the more sensitive esters, is less than 1 % when it is kept for 1 hour at 132°, but it is about 10 % at 156° under the same working conditions.

RESULTS

Table I shows the retention volumes of several oxygen-containing terpenes; V is the relative retention volume with tetralin (taken as 1) as the reference standard and V_g the retention volume relative to the absolute retention volume of tetralin corrected for the pressure drop and the column temperature; this absolute retention volume was found to be 404 ml/g for silicone oil and 238 ml/g for Hyprose S.P. 80.

In Fig. 1 the logarithm of the retention volume of several oxygen-containing

terpenes is plotted against the boiling point; it can be observed that the behaviour of most terpenes is quite different with the two stationary phases used. With silicone oil all terpenes are eluted according to their boiling points, with Hyprose the order

TABLE I

RETENTION VOLUME OF OXYGEN-CONTAINING TERPENES

Substance	*Silicone D.C. 550*		*Hyprose S.P. 80*	
	V	V_g	V	V_g
Tetralin (reference)	1.00	404.0	1.00	238.0
Alcohols				
Cineole	0.38	153.5	0.33	78.5
Linalool	0.56	226.2	1.29	307.0
Menthol	0.99	400.9	2.63	626.0
α-Terpineol	1.10	444.4	3.37	802.0
Citronellol	1.15	464.6	4.48	1066.2
Nerol	1.41	570.6	5.32	1266.1
Geraniol	1.48	598.0	6.65	1583.7
Eugenol	3.41	1378.6		
Carbonyl compounds				
Caproic aldehyde	0.32	129.2	0.43	102.3
Methylheptenone	0.53	214.1	0.55	131.0
Caprylic aldehyde	0.57	230.2	0.67	159.4
Citronellal	0.76	307.0	1.14	271.3
Capric aldehyde	1.01	408.0	1.05	250.0
β-Citral (Neral	1.52	614.0	2.94	699.7
α-Citral (Geranial)	1.85	757.4	3.63	863.9
Carvone	1.74	703.0	3.81	907.7
Cumic aldehyde	1.76	711.0	3.45	821.1
Cinnamaldehyde	2.70	1090.8	9.98	2376.2
Esters				
Linalyl acetate	1.34	541.3	1.24	295.1
Geranyl acetate	2.95	1191.8	2.98	709.2

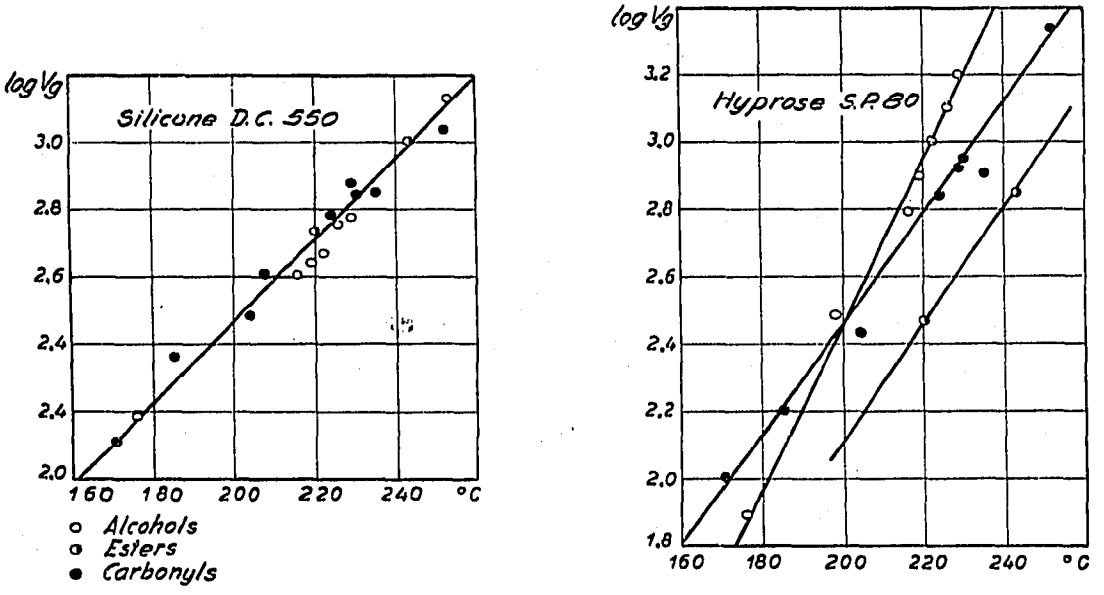

Fig. 1. Log of retention volume *vs.* boiling point for oxygen-containing terpenes on a Silicone column and a Hyprose column.

of elution differs according to the nature of the functional group. The alcohols are more strongly retained than the aldehydes and esters, while terpene hydrocarbons have a very low affinity to Hyprose so that their elution occurs very quickly.

In the analysis of mixtures of terpenes and of essential oils the use of columns with quite different properties is particularly effective for achieving good separations and for the identification of the nature and the functional group of a certain terpene.

In Fig. 2 the logarithm of the retention volume obtained on a Silicone column is plotted *vs.* the logarithm of that on a Hyprose column: with a fair approximation most of the values lie on straight lines.

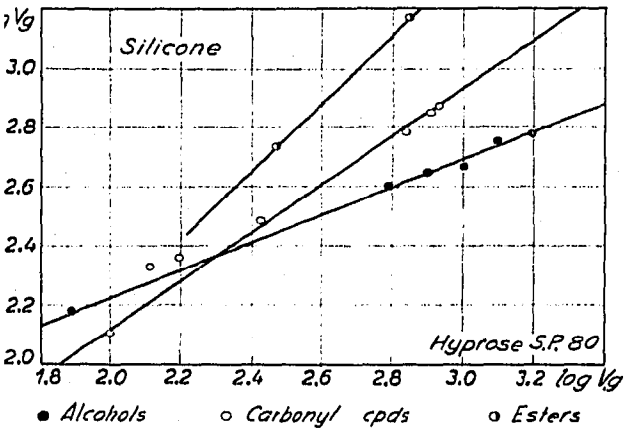

Fig. 2. Determination of the nature of oxygen-containing terpenes by comparing their behaviour on two columns, Silicone D.C. 550 and Hyprose S.P. 80.

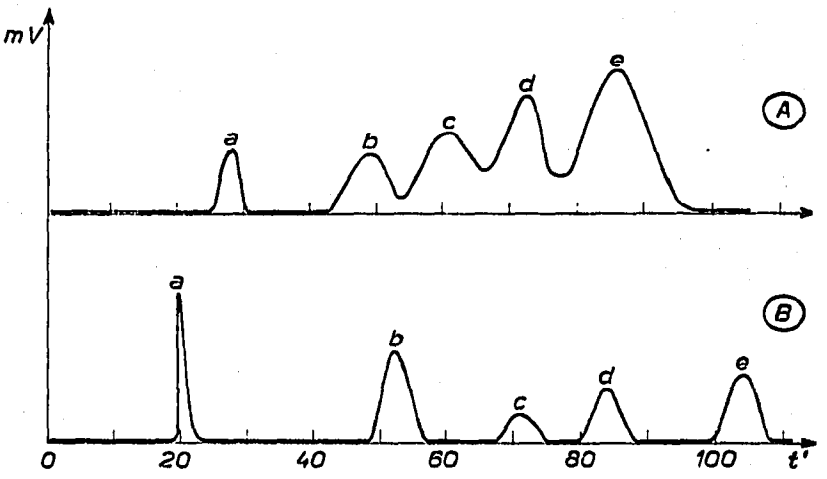

Fig. 3. Gas chromatogram of a mixture of linalool (a), terpineol (b), citronellol (c), nerol (d), and geraniol (e). $t = 132°$, $p = 2$ atm, gas flow-rate 110 ml/min. (A) 3 m column of Celite–Silicone 20%; (B) 3 m column of Celite–Hyprose S.P. 80 20%.

In Fig. 3 one of the more interesting applications of the use of the Hyprose column is presented. It shows the chromatograms of terpene alcohols of similar structure obtained on silicone (A) and Hyprose columns (B); with the latter a complete separation is achieved.

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SUMMARY

The separation and identification of oxygen-containing terpenes can be performed by gas chromatography. The operating temperature should be low (< 160°), the gas flow-rate high (~ 100 ml/min), and columns of high resolution (> 3000 HETP) with stationary phases having quite different properties should be used. With a strongly polar phase (Hyprose S.P. 80) terpene alcohols can be very effectively separated.

REFERENCE

1 A. LIBERTI AND G. P. CARTONI, *Ricerca sci.*, 28 (1958) 1192.