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RELATIVE RETENTION TIME CHANGES WITH TEMPERATURE FOR THE GAS CHROMATOGRAPHIC IDENTIFICATION OF VOLATILE OIL COMPONENTS

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SUMMARY

Changes in relative retention times of volatile oil components with change in column temperature appear to relate to polarity matches of the substance concerned with the stationary phase used. Usually relative retention times to linalol decrease with increase in temperature, but this is not always so. Such observations, on three different types of stationary phase, seem to be of value in indicating the class of oil constituents.

INTRODUCTION

Discussing gas chromatographic relative retention times, CRAMERS AND KEULEMANS[1] have observed that these are affected "only slightly by the column temperature" and that their dependence on temperature "is complicated, no general rules can be given". In the course of a study of constituents of volatile oils, we have observed general patterns of relative retention times which should prove of value in identifying the class of an unknown oil constituent.

EXPERIMENTAL

A Beckman "GC-M Research" gas chromatograph with FID, and Sargent recorder was used. Nitrogen or helium was used as mobile phase, supplied at 60 ml/min, with hydrogen supplied to the detector at 92.5 ml/min to give maximum sensitivity. Three different stainless steel columns were used, each 2 m long × 5 mm I.D., packed by the manufacturer with 15% stationary phase on 42/60 Chromosorb W. Stationary phases used were the non-polar dimethyl polysiloxane (SE-30), the increasingly polar diethylene glycol succinate (DEGS), and the polyethyleneglycol Carbowax 20M. Reference volatile oil constituents and oils were obtained from various commercial sources, including some kindly presented by Plaimar Ltd. of Perth, W.A. Relative retention times were observed against linalol as standard on several different occasions

TABLE I

RELATIVE RETENTION TIMES OF SUBSTANCES TO LINALOL ON GIVEN STATIONARY PHASES AT VARIOUS TEMPERATURES

Stationary phase	*Column temperature (isothermal)*					
	160°	*175°*	*190°*	*205°*	*220°*	
	Citronellyl acetate (A1)					
DEGS	1.00	1.00	1.00	1.00	1.00	
20 M	1.24	1.20	1.18	1.17	1.11	
SE-30	2.73	2.54	2.31	2.20	2.08	
	Geranyl acetate (A1)					
DEGS	1.58	1.57	1.55	1.50		
20 M	1.83	1.74	1.71	1.64	1.56	
SE-30	3.05	2.79	2.61	2.38	2.21	
	Linalyl acetate (A1)					
DEGS	1.08	1.04	1.00	1.00	1.00	
20 M	1.14	1.15	1.13	1.13	1.12	
SE-30	1.98	1.87	1.78	1.73	1.65	
	Menthyl acetate (A1)					
DEGS	1.15	1.14	1.14	1.13	1.12	
20 M	1.31	1.33	1.35	1.35	1.35	
SE-30	2.52	2.33	2.22	2.10	2.00	
	Terpinyl acetate (A1)					
DEGS	2.05	2.00	1.91	1.88	1.83	
20 M	2.14	2.15	2.15	2.13	2.13	
SE-30	3.20	2.90	2.83	2.60	2.43	
	Cineole (A2)					
DEGS	0.26	0.31	0.32	0.33	0.38	
20 M	0.31	0.36	0.39	0.47	0.42	
SE-30	0.86	0.86	0.90	0.91	0.91	
	Isomenthone (A2)					
20 M	0.97	1.03	1.07	1.09	1.13	} note!
DEGS	1.00	1.07	1.09	1.14	1.17	
SE-30	1.39	1.38	1.39	1.39	1.39	
	Limonene (A2)					
DEGS	0.21	0.24	0.26	0.28	0.33	
20 M	0.25	0.31	0.33	0.35	0.40	
SE-30	0.76	0.80	0.85	0.87	0.82	
	Myrcene (A2)					
DEGS	0.21	0.23	0.24	0.25	0.33	
20 M	0.21	0.24	0.27	0.30	0.30	
SE-30	0.55	0.56	0.56	0.57	0.54	
	β-Pinene (A2)					
DEGS	0.16	0.17	0.18	0.24	0.25	
20 M	0.18	0.23	0.25	0.27	0.31	
SE-30	0.65	0.68	0.73	0.73	0.70	
	Citronellol (B1)					
SE-30	1.79	1.71	1.67	1.56	1.47	
DEGS	2.16	2.06	2.00	1.88	1.83	
20 M	2.58	2.48	2.24	2.17	1.94	
	Geraniol (B1)					
SE-30	2.01	1.93	1.83	1.76	1.65	
DEGS	3.21	3.06	2.91	2.63	2.49	
20 M	3.62	3.35	3.10	2.91	2.72	

TABLE I (*continued*)

Stationary phase	*Column temperature (isothermal)*					
	160°	*175°*	*190°*	*205°*	*220°*	
	Isoborneol (B1)					
SE-30	1.50	1.50	1.49	1.47	1.47	
DEGS	1.83	1.85	1.80	1.73		
20 M	1.90	1.91	1.89	1.91	1.88	
	Menthol (B1)					
SE-30	1.54	1.48	1.45	1.43	1.40	
DEGS	1.48	1.50	1.48	1.47	1.50	
20 M	1.63	1.64	1.63	1.64	1.63	
	Anethole (B2)					
SE-30	2.28	2.17	2.12	2.03	1.95	
20 M	3.53	3.44	3.26	3.17		
DEGS	4.02	3.75	3.55	3.38	3.17	
	Cinnamaldehyde (B2)					
SE-30	2.29	2.24	2.15	2.03	1.92	
20 M			6.9	6.4	5.9	
DEGS		10.00	9.35	8.65	8.0	
	Estragole (B2)					
SE-30	1.61	1.55	1.52	1.47	1.45	
20 M	2.00	1.94	1.92	1.92	1.89	
DEGS	2.25	2.15	2.11	2.05	2.00	
	Eugenol (B2)					
SE-30	3.29	3.09	2.83	2.67	2.51	
20 M			9.8	9.0	7.6	
DEGS		12.5	11.3	10.3		
	Safrole (B2)					
SE-30	2.25	2.19	2.13	2.04		
20 M	4.21	4.04	3.94	3.82	3.59	
DEGS		4.88	4.65	4.25		
	Camphor (B3)					
20 M	1.24	1.31	1.35	1.36	1.38	}note!
SE-30	1.39	1.41	1.41	1.41	1.39	
DEGS	1.47	1.55	1.61	1.63	1.66	
	Carvone (B3)					
SE-30	2.05	2.00	1.90	1.83	1.73	
20 M	2.67	2.65	2.55	2.54	2.38	
DEGS	3.22	3.13	3.09	2.93		
	Citral (B3)					
SE-30	2.21	2.07	1.97	1.86	1.73	
20 M	2.46	2.42	2.31	2.26	2.16	
DEGS	2.89	2.79	2.75	2.63		
	Piperitone (B3)					
SE-30	2.12	2.04	1.97	1.93	1.86	
20 M	2.62	2.63	2.63	2.64	2.63	
DEGS	3.11	3.03	3.00	2.87		
	Pulegone (B3)					
SE-30	1.95	1.87	1.88	1.86	1.77	
20 M	1.95	2.00	2.00	2.00	2.00	
DEGS	2.17	2.16	2.20	2.19	2.17	
	Terpineol (B3—anomalous)					
SE-30	1.62	1.58	1.56	1.53	1.50	
20 M	2.11	2.06	2.00	1.95	1.89	
DEGS	2.16	2.13	2.10	2.07	2.00	

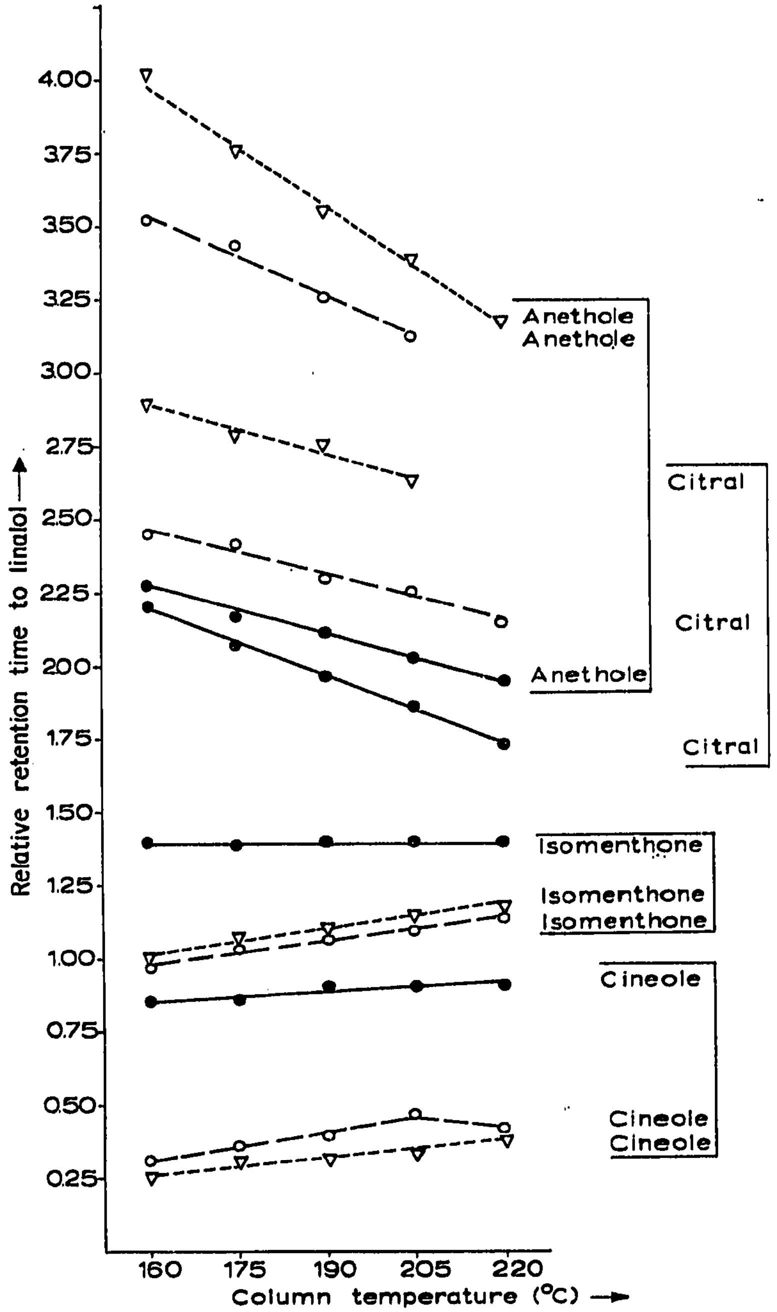

Fig. 1. Relative retention times to linalol on three columns at different column temperatures. ▽— — —▽, on DEGS; ○— — —○, on 20 M; ●—●, on SE-30.

for the various oil constituents, at a series of isothermal temperatures 15° apart, the oven temperature being checked by thermistor. The upper temperature limit was the limitation of the column stationary phase material, and the lower limit, the reasonable time for obtaining peaks.

RESULTS AND DISCUSSION

Relative rather than absolute retention times were used for this work, to offset

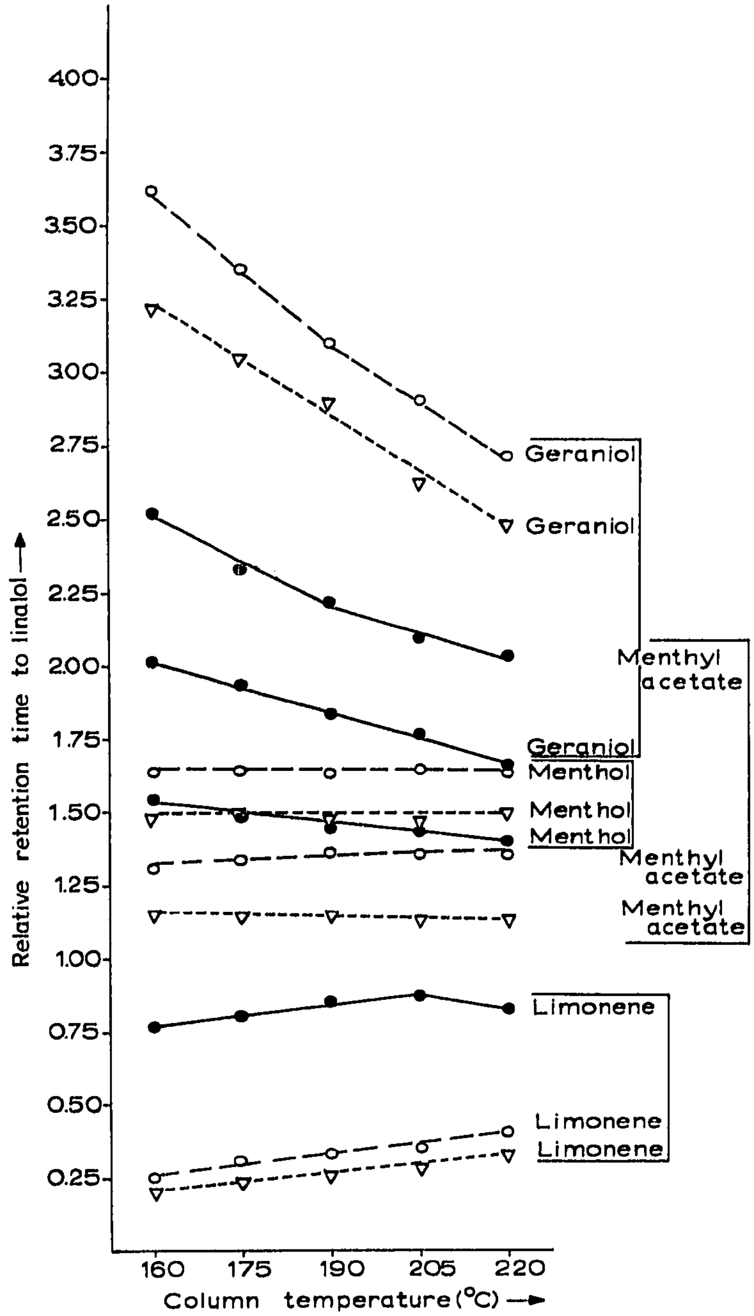

Fig. 2. Relative retention times to linalol on the three columns indicated, at different column temperatures. ▽— — —▽, on DEGS; ○— — —○, on 20 M; ●—●, on SE-30.

any slight day-to-day changes in mobile phase flow rate. They were calculated from chart distances measured in millimeters. Linalol was chosen as the standard as it has a fairly short retention time (between 0.9 min on Carbowax 20M at 220°, and 6.0 min at 145°) intermediate between those of the non-polar terpene hydrocarbons such as limonene, and the slightly polar oxygenated terpenoids such as carvone, which represent the most frequently occurring components of many volatile oils.

Results are arranged according to polarity in Table I in five groups, which are explained below, and some results are plotted in Figs. 1 and 2 to illustrate graphically

TABLE II

RELATIVE RETENTION TIMES OF SOME SUBSTANCES TO LINALOL ON GIVEN STATIONARY PHASES AT 205° WITH DIFFERENT MOBILE PHASES

Mobile phase	*Stationary phase*		
	SE-30	*20 M*	*DEGS*
	Menthol		
Helium	1.43	1.66	1.43
Nitrogen	1.44	1.63	1.50
	Piperitone		
Helium	1.93	2.66	2.86
Nitrogen	1.94	2.63	2.88

the patterns characteristic of the groups, which serve to identify members of each of them. Change of mobile phase produced very little change in relative retention time (see Tables II and III) unless high temperatures, near the limit for the stationary phase, were used. Thus results obtained with the nitrogen and helium are averaged in Table I. Whilst the range of stationary phases available for columns is very great, those used represent the three main types, namely non-polar (SE-30), slightly polar electron-donor (DEGS), and more polar electron-acceptor (20M), and so should indicate the style of result which would be obtained using other columns of these types. For all substances examined, of course, the actual retention time decreased with increase in temperature of the column. When measured against the standard linalol, a relatively polar substance, the relative retention times also decreased with increase in temperature, except in the case of some non-polar compounds.

For most slightly polar substances, the relative retention times to linalol are greatest on DEGS, of the three stationary phases used. This includes terpenoid carbonyls such as citral and substituted aromatics such as anethole (see Fig. 1). Their limited polarity thus appears to be best matched by the limited polarity of DEGS. The only exception to this is that for most terpenoid alcohols such as geraniol, the relative retention times shown on 20M are slightly greater than those shown on DEGS, indicating the higher polarity of these alcohols, best matched by the 20M glycol

TABLE III

RELATIVE RETENTION TIMES OF SOME SUBSTANCES TO LINALOL ON CARBOWAX 20 M GLYCOL AT VARIOUS TEMPERATURES WITH DIFFERENT MOBILE PHASES

Mobile phase	*Column temperature (isothermal)*				
	160°	*175°*	*190°*	*205°*	*220°*
	Piperitone				
Helium	2.63	2.63	2.59	2.66	2.56
Nitrogen	2.61	2.63	2.63	2.63	2.63
	Safrole				
Helium	4.21	4.00	3.94	3.83	3.56
Nitrogen	4.22	4.08	3.94	3.82	3.63

groups (see Fig. 2). Nevertheless, in almost all cases where the solute can be said to be slightly polar (as in the B group of Table I) relative retention times are lowest on the non-polar SE-30 column at a given temperature.

The lowering of the polarity of terpenoid alcohols upon esterification (*e.g.* menthol to menthyl acetate) is clearly indicated by the results obtained (placing them in Group A of Table I) in which an inversion of response to polar and non-polar stationary phases is observed, although their polar "origin" is perhaps indicated by the slope of these plots, when compared with other non-polar substances, such as limonene (see Fig. 2). In contrast to the slightly polar substances, the non-polar volatile oil components exhibit their highest relative retention times on SE-30 (usually with the lowest values on DEGS). These non-polar substances fall into two groups, the terpenoid esters, in which relative retention time, as usual, decreases with rise in column temperature, and the terpene hydrocarbons (*e.g.* limonene), in which it increases slightly or remains constant.

To summarise the information for identifying the chemical class of an unknown component of a volatile oil using its relative retention times to linalol:

A. Non-polar substances with relative retention times on SE-30 greater than those on both the other two columns: (1) Relative retention times decrease rapidly on SE-30 as temperature rises—terpenoid ester. (2) Relative retention times are low and increase slightly as temperature rises—terpene hydrocarbon or non-polar terpenoid.

B. Slightly polar substances with relative retention times on SE-30 below those on the other two columns, or at least not greater than both: (1) Relative retention times greatest on 20M—terpenoid alcohol. (2) Relative retention times greater on DEGS than on 20M, but with the results on these two polar columns fairly close, converging as temperature rises, and both well-separated from those on SE-30—substituted aromatic. (3) Relative retention times greater on DEGS than on 20M, with those on 20M roughly equally spaced between the other two—terpenoid carbonyl.

The results given for limonene are typical for group A2 substances such as β-pinene, cineole and myrcene as well. Thus the low polarity of the terpenoid ether cineole is comparable to that of terpene hydrocarbons. The non-polar column (SE-30) is clearly best for distinguishing between these substances. The saturated terpenoid carbonyl isomenthone is also sufficiently low in polarity to fall in this group, although its relative retention time to linalol is approximately 1.40. Typically for this group A2, the relative retention times for isomenthone on the two polar columns increase with rising temperature (Fig. 1), so isomenthone changes from emerging ahead of linalol to being behind it on the column.

Menthol, in contrast to isomenthone, is sufficiently polar to fall with other terpenoid alcohols into group B1, with results for SE-30 and DEGS close together, and, in fact, crossing, being just less than those on 20M. The slopes of the results for isomenthone, which in two cases increase with temperature rise, are again unlike those of menthol (Figs. 1 and 2). Isoborneol shows similar behaviour to menthol. The acyclic terpenoid alcohols geraniol and citronellol exhibit distinctly higher relative retention times than menthol and isoborneol, especially on the polar columns, and show rapid decrease in values as temperature increases. The only terpenoid alcohol not appearing with the others in group B1 is terpineol, for although terpineol results on polar columns are distinctly greater than those on SE-30, the values on 20M are not quite above those for DEGS. Other terpenoid alcohols examined all show highest relative retention times on the 20M glycol column, unlike all other substances examined here, including the phenolic alcohol eugenol.

Camphor, whilst showing similar relative retention times on SE-30 to isomenthone, menthol and isoborneol, falls satisfactorily into the appropriate group B3 of carbonyl terpenoids. Camphor is the only substance examined for which values on SE-30 come between those on DEGS (greatest) and 20M, and the rising slope of the results obtained on polar columns suggests an affinity with the low-polarity substances such as cineole. At higher temperatures than those possible here, it appears from extrapolation that the more usual situation would occur with results on SE-30 being the lowest.

Results obtained with menthyl acetate are typical of those for all the other terpenoid acetate esters—perhaps surprisingly in the case of terpinyl acetate in view of the anomalous behaviour of terpineol. Values for citronellyl and linalyl acetates on DEGS are very close to unity.

The terpenoid carbonyls in group B3 such as citral give distinctive results in that plots of the relative retention times show 20M in the middle with DEGS results approximately as far above as those on SE-30 are below the 20M figures. From this distribution the carbonyls are thus less polar than the terpenoid alcohols but more polar than the esters. Carvone and piperitone are normally difficult to distinguish by gas chromatography, but piperitone, unlike carvone, hardly alters in relative retention time to linalol on 20M, as the temperature is changed.

The substituted aromatics of group B2 are also distinct because of the converging tendency of their values on the two polar columns as the temperature rises, these normally being widely separated from the non-polar column results. The distribution of these results, however, suggests that they are only comparable in polarity with the carbonyls of group B3. Substances such as apiole, which fall in group B2, exhibit relative retention times of up to 18 times that of linalol on DEGS, and such values tend to be inconvenient and unreliable for identification work. Thus, although on grounds of matching polarity DEGS or 20M columns give the best separation of substituted aromatics, for speedy results the non-polar SE-30 column is to be preferred. These high retention times on polar columns do not, however, mean that the group B2 compounds are more polar than those of group B1, for the results on DEGS and 20M suggest the reverse.

From the polarity match principle, indicated by a substance exhibiting greatest relative retention times on the column stationary phase most closely resembling it in polarity, terpene hydrocarbon and terpenoid ester oils should be chromatographed on non-polar columns, terpenoid alcohol oils on polar glycol columns, and oils with terpenoid carbonyls or aromatics (not too highly substituted) on slightly polar ester columns. However, as volatile oils are complex mixtures in many cases, one type of column is likely to be inadequate for best separation of all the oil components.

REFERENCE

1 C. A. M. G. Cramers and A. I. M. Keulemans, in J. Krugers (Editor), *Instrumentation in Gas Chromatography*, Centrex, Eindhoven, 1968.