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ANALYSIS OF STRAIGHT-CHAIN TERPENE ALCOHOLS BY GAS CHROMATOGRAPHY

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SUMMARY

The parameters affecting the separation and quantitation of geraniol, nerolidol, farnesol, geranyl geraniol, phytol, and their trimethylsilyl ethers and acetates have been investigated. The characteristics of the partially reduced derivatives of geranyl geraniol have been compared with those of phytol and of the fully reduced derivative phytanol. 3% SE-30 on Gas-Chrom Q was found suitable and gave no degradation of the free alcohols. For the homologous series geraniol, farnesol and geranyl geraniol log retention volume was proportional to carbon number. For all compounds log retention volume was inversely proportional to the absolute temperature. A Double Bond value has been defined and found useful in describing the efficiency of a column when used for separating geranyl geraniol from its reduced derivatives.

INTRODUCTION

Identification and quantitative analysis of straight-chain terpene alcohols (terpeneols) is of importance in the study of the biosynthesis of carotenoids, gibberellins, sterols, and of the phytol moiety of chlorophyll. Procedures for the gas chromatographic (GC) analysis of geraniol, farnesol and geranyl geraniol as the free alcohols have been reported[1–3] and a sensitive method for the detection of phytol has been published[4]. The analysis of both geranyl geraniol[3] and phytol[4] was hampered by the degradation of the alcohols on the chromatographic column.

In order to improve the methods currently available, a systematic investigation of the GC characteristics of these compounds as their free alcohols and as their trimethylsilyl (TMS) ethers and acetates has been carried out. Chromatography of the partially reduced derivatives of geranyl geraniol has been compared with that of phytol and of the fully reduced derivative phytanol.

MATERIALS AND METHODS

Terpene alcohols

Phytol (>95% pure) was obtained from Sigma Chemical Co., London, Great

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Britain; geraniol, nerolidol, farnesol, and squalene (all >90% pure) were obtained from Koch-Light Laboratories, Colnbrook, Great Britain. Geranyl geraniol was a gift of Professor O. Isler of Hoffman-La Roche, Basle, Switzerland. All-*trans*-geranyl geraniol was purified from a mixture of the all-*trans* and *cis*–*trans* isomers by thin-layer chromatography (TLC) on silica gel H plates, 1 mm thick, developed with light petroleum (b.p. 40–60°)–diethyl ether (7:3). A mixture of analogues of geranyl geraniol and phytol was prepared by partially reducing geranyl geraniol over 10% palladium on calcium carbonate. Phytanol of 97% purity was prepared by hydrogenation of phytol.

Preparation of derivatives

TMS ethers were prepared by reaction of the alcohols with bis(trimethylsilyl) acetamide at room temperature. The reaction was complete in 1 min but generally 5–10 min were allowed. The terpeneol acetates were prepared by reaction of the alcohols with acetic anhydride dissolved in an equal volume of pyridine. An attempt was made to prepare the trifluoroacetates from trifluoroacetic anhydride, previously obtained by the distillation of trifluoroacetic acid (Hopkin & Williams, Chadwick Heath, Great Britain) over P_2O_5, by reaction with the free alcohols. However, mainly degradation products were produced.

Gas chromatography

The stationary phase materials SE-30 (methyl polysiloxy gum), or QF-1 (fluoroalkyl silicone oil) on Gas Chrom Q 100–120 mesh, were purchased from Applied Science Labs., State College, Pa., U.S.A.; oxygen-free nitrogen, argon, air, and hydrogen were obtained from the British Oxygen Co., Wolverhampton, Great Britain. GC was carried out using either a Pye 204 dual-column chromatograph with flame ionisation detectors or a Pye Panchromatograph fitted with an argon ionisation detector (Pye-Unicam, Cambridge, Great Britain). Nitrogen and argon flow-rates of 40 ml/min were employed.

Choice of columns

In order that separation of high-molecular-weight terpenoids formed from the terpeneols could also be achieved, the stationary phases SE-30 and QF-1 used earlier for triglycerides[5] were chosen. However, the problem of degradation had to be overcome. Liljenberg and Odham[4] found it possible to chromatograph free phytol on 2% hyprose SP-80 (octakis-(2-hydroxypropyl)sucrose) but found that SE-30 and Versamid 900 gave complete degradation at all per cent loadings. Nevertheless, it was found that both 3% SE-30 or 3% QF-1 on Gas-Chrom Q, 100–120 mesh, packed in 168 cm × 6.5 mm glass columns, were suitable. After conditioning overnight, at 350 °C and 250 °C for SE-30 and QF-1, respectively, up to five injections of 1 mg phytol were applied. Applications of other terpenes or triglycerides were also effective in stopping degradation.

As for the free alcohols on Apiezon L and poly(ethyleneglycol adipate)[1], no specific interactions with respect to double bonds or isomers were observed between any of the terpeneols or their derivatives and the stationary phases. Thus apart from there being less tailing on 3% SE-30 compared with 3% QF-1, there was very little difference in chromatographic behaviour between the two phases. Since SE-30 is

stable at higher temperatures than QF-1, results are recorded for the SE-30 column only.

RESULTS

Isothermal separation

The separation of a mixture of terpeneols and some derivatives under conditions where log V_r was approximately constant for both parent compounds and derivatives is shown in Fig. 1 (V_r = retention volume). Fig. 2 shows the separation of phytol, its TMS ether and acetate, together with an analysis of the non-saponifiable lipid fraction of the leaves of the French bean *Phaseolus vulgaris*. Fig. 3 illustrates the effect of temperature on log V_r for all-*trans*-nerolidol, phytol, and derivatives. The elution of all these compounds conformed to the relationship $\log V_r = a + b/T$, where a and b are constants and T is in °K. There was, however, very little difference in the elution volume of nerolidol and its derivatives. Log V_r was also proportional to the number of carbon atoms (C) in geraniol, farnesol and geranyl geraniol at all the temperatures stated (Fig. 4). The relationship $\log V_r = a + b/T$ was again obeyed by the three homologues and is shown in Fig. 5. The relationship between boiling point and log V_r is plotted in Fig. 6. For convenience, Fig. 6 also shows the relationship between boiling

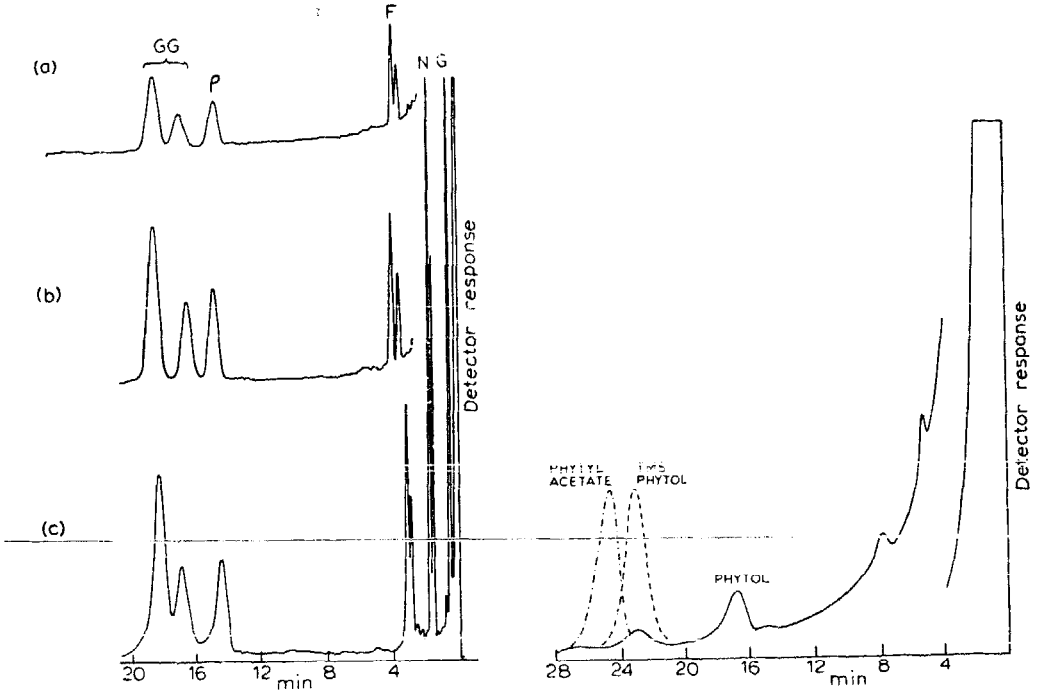

Fig. 1. Comparative GC of (a) free terpeneols at 190 °C, (b) TMS ethers at 195 °C, and (c) acetates at 199°C on 3% SE-30. G = geraniol; N = nerolidol; F = farnesol; P = phytol; GG = geranyl geraniol.

Fig. 2. GC of the non-saponifiable fraction of an extract from 12-day-old *Phaseolus vulgaris* leaves, low in chlorophyll. The positions of TMS phytol and phytyl acetate are also indicated.

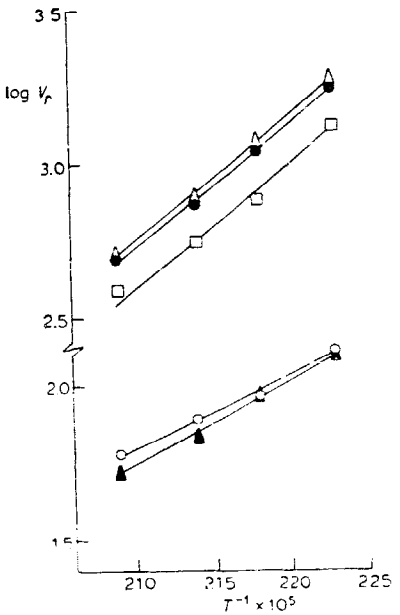

Fig. 3. Relationship between log V_r and $1/T$ for phytyl acetate (○), TMS phytol (●), phytol (□), all-*trans*-nerolidol TMS ether (▯), and all-*trans*-nerolidol and its acetate (▲).

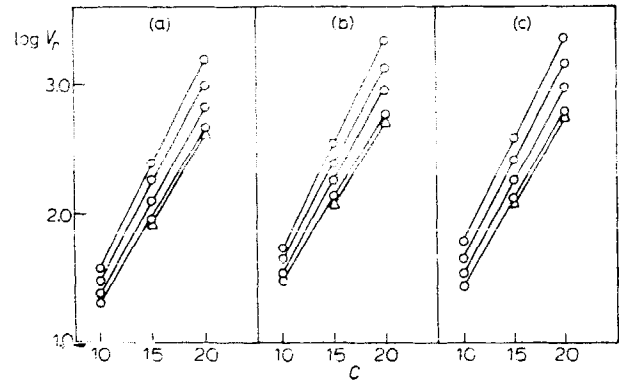

Fig. 4. Relationship between log V_r and C at various temperatures for geraniol, farnesol and geranyl geraniol free terpenols (a) TMS ethers (b), and acetates (c). ○, all-*trans* isomers; △, *cis-trans* isomers. Though only plotted for 205 °C isomers could be separated at all temperatures.

point and the relative elution temperature (T_{RE}) index of temperature programmed analyses.

Resolution

Fig. 1 illustrates qualitatively the differences in resolution obtained when the terpeneols or some derivatives were used for the analysis. Table 1 gives the efficiencies of SE-30 for the C_{20} terpenoids under these conditions, both in terms of theoretical plates (TP) or "1 Double Bond". Our definition of the latter parameter is analogous

to ΔC defined for triglycerides[9]. It is an empirical value which indicates the difference in the number of double bonds for any two all-*trans*-C_{20} terpeneols between phytol (which contains one double bond per molecule) and geranyl geraniol (which contains four double bonds per molecule) necessary for complete resolution. Thus, if a column is to separate completely the four unsaturated analogues, a Δ Double Bond value of $\leqslant 1$ is required; if only a separation of phytol and geranyl geraniol is needed then a Δ Double Bond value of $\leqslant 3$ is adequate. The maximum number of peaks (M) which can be completely separated in a particular chromatogram is given by

$$M = \frac{2\Delta t}{B_x + B_y}$$

where Δt is the difference in retention times of reference compounds x and y and B_x and B_y are the baseline peak widths of compounds x and y, respectively.

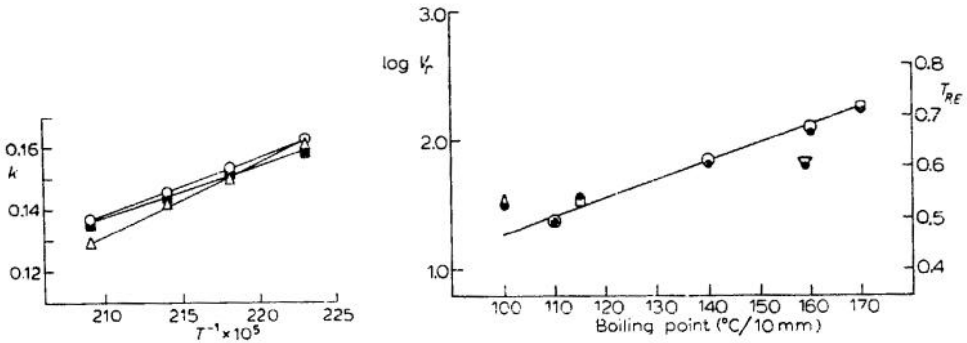

Fig. 5. Relationship between k and $1/T$ for geraniol, farnesol and geranyl geraniol. ○, Free terpeneols; △, TMS ethers; ■, acetates. k is the constant of log $V_r = kC - N$.

Fig. 6. Relationship between boiling point (°C/10 mm) and log V_r [195°]. ○, Geraniol, nerolidol and farnesol; □, geranyl and farnesyl acetates; △, TMS geraniol; ▽, nerolidyl acetate; ●, relative elution temperatures (T_{RE}) superimposed for comparison. Boiling points have been taken or extrapolated from data in the literature[6-8].

TABLE I

COLUMN EFFICIENCIES OF SE-30 FOR PHYTOL AND GERANYL GERANIOL, AND FOR THEIR DERIVATIVES

Conditions were those in Fig. 1. Determinations were in duplicate. TP is total number of theoretical plates of the column. Δ Double Bond is the minimum difference in the number of double bonds between two C_{20} terpeneol analogues necessary for complete resolution in the phytol–geranyl geraniol region of the chromatograms.

Compound	*TP*	Δ *Double Bond*
Phytol	2330 ± 15	2.4 ± 0.1
Geranyl geraniol	2695 ± 10	
Phytol TMS ether	2865 ± 35	1.6 ± 0.1
Geranyl geranyl TMS ether	3175 ± 5	
Phytyl acetate	2830 ± 20	1.5 ± 0.1
Geranyl geranyl acetate	3080 ± 45	

Since the double bond difference between phytol and geranyl geraniol is 3

$$\Delta \text{ Double Bond} = \frac{3}{M} = \frac{1.5(B_{GG}+B_P)}{\Delta t}$$

where B_{GG} is the baseline peak width of geranyl geraniol or its derivative and B_P is the baseline peak width of phytol or its derivative.

The relationship between total theoretical plates (n) of a column and Δ Double Bond is also simple to derive:

$$\Delta \text{ Double Bond} = \frac{1.5(B_{GG}+B_P)}{\Delta t} = \frac{1.5(B_{GG}+B_P)}{(t_{GG}-t_P)}$$

since

$$n = 16\left(\frac{t_r}{W}\right)^2$$

then

$$n_{GG} = 16\left(\frac{t_{GG}}{W_{GG}}\right)^2 \text{ and } n_P = 16\left(\frac{t_P}{W_P}\right)^2$$

$$\therefore \frac{1.5(B_{GG}+B_P)}{(t_{GG}-t_P)} = \frac{1.5(B_{GG}+B_P)}{\sqrt{n_{GG}}\cdot W_{GG}/4-\sqrt{n_P}\cdot W_P/4} = \frac{6(B_{GG}+B_P)}{\sqrt{n_{GG}}\cdot W_{GG}-\sqrt{n_P}\cdot W_P}$$

where

t_r, W = generalised retention time and base width between tangents of a Gaussian peak

n_{GG}, n_P = total number of theoretical plates of column with reference to geranyl geraniol and phytol

W_{GG}, W_P = distance between peak tangents at baseline for geranyl geraniol and phytol.

t_{GG}, t_P = retention time of geranyl geraniol and phytol.

The actual resolving power of the 3% SE-30 column is shown in Fig. 8, where the acetates and TMS ethers of the products of partial hydrogenation of geranyl

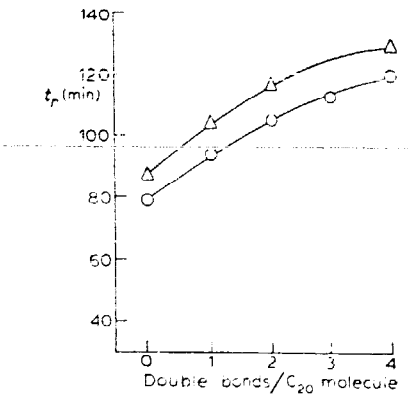

Fig. 7. The relationship between the number of double bonds in geranyl geraniol and reduced derivatives, and retention time, t_r (min). ○, TMS ethers; △, acetates.

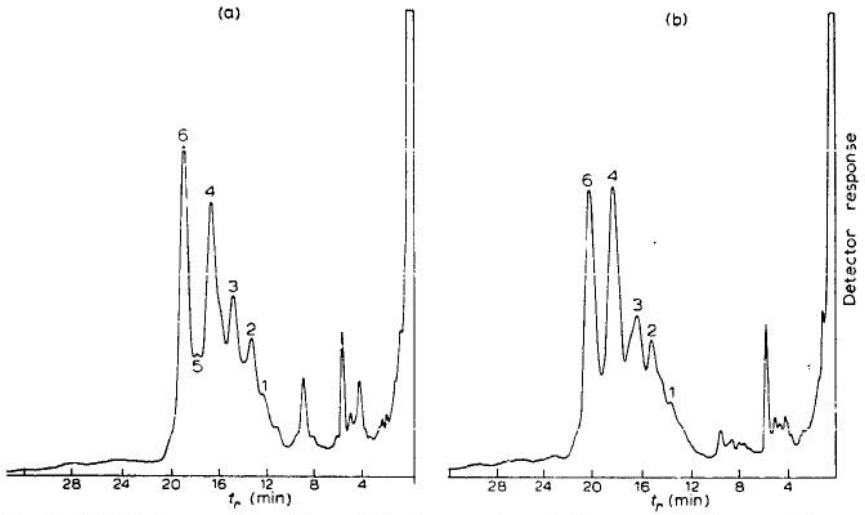

Fig. 8. GC of the products of partial hydrogenation of all-*trans*-geranyl geraniol TMS ethers (a) and acetates (b). 1 = Phytanol; 2 = *cis*-phytol; 3 = phytol; 4 = *cis*–*trans*-geranyl geraniol + all-*trans*-tetrahydrogeranyl geraniol; 5 = all-*trans*-dihydrogeranyl geraniol; 6 = all-*trans*-geranyl geraniol.

geraniol have been chromatographed. Fig. 7 shows the plot of retention time, t_r, against number of double bonds per molecule for both acetates and TMS ethers.

Temperature programmed analysis

From experience, a convient programme was 110–300°C at 3.71°C/min. (The dial setting of the programmer was 4°C/min.) This allowed squalene to be used as internal standard, since its elution temperature is 270° ± 1°C. The relative elution temperatures (T_{RE})[10] of the terpeneols are given in Table II. A plot of T_{RE} *versus* boiling point is shown in Fig. 6.

The carbon equivalent of a TMS group (ΔC_{TMS})[11] and the analogous carbon equivalent of an acetate group (ΔC_{AC}) are shown in Table III.

Quantitative analysis

As expected, detector response was linear with loading, up to 20 μg per peak and the plot did not pass through the origin.

DISCUSSION

Choice of derivative

It has been reported[12,13] that trifluoroacetylation of saturated and mono-unsaturated alcohols gave derivatives which were more volatile than the parent compounds. Unfortunately, trifluoroacetylation of the terpeneols led to degradation of the parent compounds. Thus it must be assumed that in any compound where there is unsaturation and an active methylene group[13], trifluoroacetylation will lead to degradation. As reported for the fatty alcohols[12,13], we found that both the terpeneol TMS ethers and the acetates were suitable derivatives for GC, having only slightly longer retention time than the parent terpeneol (Fig. 4). It was surprising to find,

however, that there was essentially no difference in retention characteristics for nerolidol and its derivatives (Figs. 3 and 6). In this case the effects on log V_r of changes in the volatility of nerolidol on forming the derivatives are counter balanced by the increase or decrease in polarity with respect to SE-30.

Isothermal analysis

For all compounds examined (Figs. 3–5), both $\log V_r = kC + N$ and $\log V_r = a + b/T$ were well obeyed. As found by Cartoni and Liberti[14] for a range of oxygenated

TABLE II

RELATIVE ELUTION TEMPERATURES (T_{RE})* OF FREE TERPENEOLS AND THEIR TMS ETHERS AND ACETATES ON SE-30

Results were in triplicate, with standard deviations given. The 3% SE-30 column was programmed at 3.71°C/min from 110–300°C.

Compound	T_{RE}		
	Free alcohol	*TMS ether*	*Acetate*
Geraniol	0.492 ± 0.006	0.527 ± 0.002	0.538 ± 0.003
cis–trans-Nerolidol	0.591 ± 0.009	0.579 ± 0.003	0.589 ± 0.002
all-*trans*-Nerolidol	0.603 ± 0.006	0.599 ± 0.004	0.603 ± 0.002
cis–trans-Farnesol	0.654 ± 0.005	0.682 ± 0.004	0.691 ± 0.003
all-*trans*-Farnesol	0.662 ± 0.005	0.695 ± 0.001	0.708 ± 0.003
Phytol	0.802 ± 0.003	0.823 ± 0.001	0.835 ± 0.002
cis–trans-Geranyl geraniol	0.809 ± 0.002	0.834 ± 0.001	0.841 ± 0.001
all-*trans*-Geranyl geraniol	0.824 ± 0.005	0.845 ± 0.002	0.851 ± 0.002

* Squalene was used as internal standard and assigned a T_{RE} of 1.00.

TABLE III

CARBON EQUIVALENTS OF A TMS GROUP (ΔC_{TMS}) AND OF AN ACETATE GROUP (ΔC_{AC}) FOR SOME TERPENEOLS ON SE-30

Temperature programmed at 3.71°C/min.
ΔC_{TMS} and ΔC_{AC} are the differences in carbon number between the derivatives and free alcohols with the same T_{RE} under the same conditions of temperature programming. Values are found by plotting the T_{RE} values of geraniol, farnesol and geranyl geraniol against carbon number, and fitting the derivative or analogue T_{RE} values to the plot.

Compound	ΔC_{TMS}	ΔC_{AC}
Geraniol	1.0	1.5
cis–trans-Nerolidol	0.3	0.0
all-*trans*-Nerolidol	0.0	0.0
cis–trans-Farnesol	1.0	1.3
all-*trans*-Farnesol	1.1	1.5
Phytol	0.7	1.0
cis–trans-Geranyl geraniol	0.8	1.1
all-*trans*-Geranyl geraniol	0.6	0.7

terpenoids on DC-550 silicone, log V_r was proportional to the boiling point for geraniol, nerolidol, farnesol and geranyl and farnesyl acetates (Fig. 6). However, both geranyl TMS ether and nerolidyl acetates deviated from this relationship, indicating more specific interactions between solute and stationary phase.

Resolution

In terms of *TP* (Table I), 3% SE-30 was more efficient in chromatographing geranyl geraniol or its derivatives than phytol and its derivatives. Since a Δ Double Bond value of $\leqslant 3$ is required (see Results) for complete separation of phytol from all-*trans*-geranyl geraniol, it can be seen from Table I that the parent alcohols and their derivatives all give very adequate Δ Double Bond values of 1.5–2.4. Though all-*trans*-geranyl geraniol is the biological precursor of phytol, chemically synthesised geranyl geraniol contains some *cis–trans* isomer as may be seen in Fig. 1. It is clear that in terms of isomer separability, the TMS derivatives are best separated. It is interesting to note the similarity of separability of free alcohols and acetates as compared to the TMS ethers. This again illustrates the difference in characteristics of volatility and relative polarity as shown in Fig. 6.

It can be seen from Fig. 8 that when the full range of unsaturation and isomers possible between all-*trans*-geranyl geraniol and phytanol are considered, TMS derivatives give slightly better resolution than acetates. However, for both derivatives, plots of t_r *versus* double bonds per molecule give smooth curves (Fig. 7). No better resolution was obtained by employing a 10% SE-30 column of the same length. A longer column would probably be needed to give total separation of isomers (Δ Double Bond $\leqslant 1$).

These conclusions may also be applied to the isomers of farnesol and nerolidol (Fig. 1). Again, the TMS ethers gave the best separations of the *cis–trans* isomers. However, it can also be seen that though isothermal analysis was satisfactory for a range of five carbon atoms difference in molecular size, temperature programming would be the method of choice for separating compounds having larger differences in carbon number.

Temperature programmed analysis

The determination of relative elution temperature (T_{RE}) as a method of defining the elution characteristics of compounds during a temperature programmed analysis has been successfully applied to the analysis of fatty acids, fatty alcohols and hydrocarbons[10] and to triglycerides[5] and mono- and diglycerides[11]. From Table II it is seen that reproducibility was very good for all compounds, indicating the general applicability of this parameter. The analogy of T_{RE} with log V_r of isothermal analysis as indicated by Schmit and Wynne[10] is well illustrated by superimposing plots of boiling point *versus* either log V_r or T_{RE} (Fig. 6). Further theoretical implications of the latter are given in ref. 15.

As for mono- and diglycerides[11], the addition of three extra methyl groups plus a silicon atom to any of the terpeneols studied here did not result in a proportional increase in carbon number. Thus ΔC_{TMS} (Table III) was found to have a maximum value of 1.1 in the case of all-*trans*-farnesol and (with the exception of nerolidol) was about one carbon atom. The formation of an acetate derivative means the addition of only two carbon atoms, and it is interesting to see that ΔC_{AC} nearly equalled the number of actual extra carbon atoms.

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