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## THE SEPARATION OF SOME TERPENOID COMPOUNDS BY GAS-LIQUID CHROMATOGRAPHY<sup>1</sup>

E. VON RUDLOFF

### ABSTRACT

A study has been made of the degree of separation of mixtures of some terpene hydrocarbons, some of their oxygenated derivatives, two sesquiterpene alcohols, and three monophenols on a variety of columns. Temperature and sample size affected the degree of separation and the solid support Chromosorb W, a calcined diatomaceous earth, was found to combine the advantages of Celite and C-22 firebrick without causing decomposition of the sample at higher temperatures. When Craig polyesters were used as liquid phases, separations equal to those obtained on polyethylene glycol were realized with the added advantage that these produced columns which are stable at 190 to 220° C. Consequently, sesquiterpene alcohols and monophenols were also separated successfully. Another useful liquid phase for both low and high temperatures was found in a meta-linked polyphenyl ether. Squalene was found to be an efficient liquid phase for the separation of terpene hydrocarbons at 130° C and lower. The possible application of the present findings for preparative work is discussed.

### INTRODUCTION

A general method for the separation of small amounts of closely related terpene hydrocarbons, some of their oxygenated derivatives as well as some higher boiling sesquiterpene alcohols and monophenols, was required in this laboratory. Such mixtures often have a narrow range of boiling points and separation by fractional distillation not only requires large amounts, but frequently fails to produce pure components. Gas-liquid chromatography (GLC) offers considerable advantages over fractional distillation when quantities of 0.01 to 0.1 g are required, and has in several instances been extended to the gram scale. This technique was, therefore, studied in some detail to find suitable conditions for complete separation of such terpenoid mixtures on a 0.1- to 1-g scale. The experiments were carried out with a Beckman GC-2 chromatograph, using helium as carrier gas, a thermal conductivity cell as detector, and 6-foot columns of 6-mm inner diameter.

Successful separations of terpenoid compounds by GLC have been described in the literature. Bernhard (1) separated five monoterpene hydrocarbons on 20-foot polyethylene glycol and *n*-octyl phthalate columns, but obtained poor results when silicone fluid, Apiezon L, or high vacuum grease was used as liquid phase. Bayer *et al.* (2) used hydrogen as carrier gas and obtained a fair degree of separation for a large variety of terpenoid compounds on columns containing silicone vacuum grease to which alkali capronates had been added. Cvrkal and Janák (3), also using hydrogen, obtained good results for

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terpene hydrocarbons on dimethylsiloxane and proposed glycerol for oxygenated compounds. The use of hydrogen as carrier gas for mixtures containing unsaturated compounds may create difficulties, since activated centers of the solid support could catalyze phases for the separation of many terpene hydrocarbons, but their retention times of 80 minutes and more are rather high. These authors discuss some of the difficulties encountered in the analysis of essential oils and point out the need for polar liquid phases which can be used at 150° C or higher to fractionate oxygenated derivatives. This has been found in the present study in adipate polyethylene glycol, succinate ethylene glycol, and phthalate ethylene glycol polyesters (Craig polyesters) and in a *m*-linked polyphenyl hydrogenation. Liberti and Cartoni (4) used silicone and tricresyl phosphate as liquid ether. Columns with polyethylene glycol were also used by Stanley (5) and Eglington *et al.* (6) and recently Westaway and Williams (7) have separated almost completely  $\alpha$ - and  $\beta$ -pinene,  $\alpha$ - and  $\gamma$ -terpinene, and limonene on a 6-foot Apiezon M column with nitrogen as carrier gas.

From the data contained in the above communications and in the monograph by Keulemans (8) it was concluded that silicone-type columns could be used for a wide range of compounds at lower and higher temperatures with a fair degree of separation. Far better separation could be expected with polyethylene glycols but the temperature limit of 100 to 130° C (8) would limit its application. These two types of liquid phases were, therefore, used to study the effect of the various physical factors involved and to compare the degree of separation obtained with polyethylene, squalene, Apiezon M, polystyrene, a polyphenyl ether, polyesters of adipic, succinic, and phthalic acids (Craig polyesters), a polyamide, and diglycerol as liquid phases. *d*-Limonene, the mixture of *p*-menthadienes produced by dehydration of  $\alpha$ -terpineol with oxalic acid as catalyst (9), and a synthetic mixture containing *d*-limonene, 1,8-cineole, fenchone, citronellal, camphor,  $\alpha$ -terpineol, citronellol, and bornyl acetate were used as test compounds. The sesquiterpene alcohols, occidentalol and eudesmol, and the monophenols, anethole, eugenol, and isoeugenol, were used for the chromatographic studies at higher temperatures.

Initial experiments in the present study showed that a number of factors influencing the separation of terpenoid compounds have not been described fully in the literature. Also, most of the liquid phases used to date for this purpose, except the rather inefficient silicone type, cannot be used above 100 to 130° C for any length of time. Furthermore, the above communications are mainly concerned with qualitative determinations and do not give the most favorable conditions for preparative separations. The present investigation was therefore extended to cover the effect of sample size, temperature, flow rate, nature of solid support, and the ratio of solid support to liquid phase, as well as a search for liquid phases which could be used successfully for the wide range required. The results obtained in this study show that sample size, temperature, and, to a lesser degree, the solid support can affect the degree of separation. Some of the liquid phases tested gave poor results when samples of 5  $\mu$ l or more were used, yet gave excellent separation with 2  $\mu$ l or less, making these columns useful for analytical work. Two types of liquid phases, Craig polyesters and a polyphenyl ether, could be used equally well at 190–220° C or at lower temperatures. An adipic acid – polyethylene glycol polyester was found to give separations equal to, or better than, those obtained on a polyethylene glycol (Carbowax 400), besides being suitable for high temperature separations, and proved to be the best general purpose column for up to 50  $\mu$ l of terpenoid compounds investigated thus far.

## EXPERIMENTAL

*Apparatus*

A Beckman GC-2 chromatograph equipped with a fraction collector (No. 92730) and a heated inlet (suitable for injection of up to 2 ml) was used in conjunction with a Bristol Dynamaster strip-chart recorder (0.05- to 1.05-mv scale). The chart speed was 0.5 inch per minute and the flow rate of helium carrier gas was measured at the exit by the soap bubble method. Samples were introduced with a Beckman liquid sampler (No. 224000) which allowed the accurate injection of 1.5 to 80  $\mu$ l quantities. The column exhaust was at atmospheric pressure (710–715 mm Hg) whereas the pressure at the column inlet was varied to give the desired flow rates.

*Solid Support and Chemicals*

Celite 545 was water-washed to remove the fines, then acid-washed, neutralized with bicarbonate solution, washed with water, and dried. C-22 Firebrick (60–80 mesh) was prepared by wet milling, drying, and sifting of commercial firebrick. Chromosorb W, a flux-calcined diatomaceous earth, produced by Johns-Manville, was obtained commercially. Silicone high vacuum grease (Dow Corning), squalene (90%, Eastman Chemicals), Carbowax 400 (Carbide and Carbon Chem. Co.), the polyphenyl ether *m*-bis(*m*-phenoxyphenoxy)-benzene (Monsanto Corp.), Apiezon M (Metropolitan-Vickers Electrical Co.), and diglycerol (Colgate-Palmolive Co.) were also obtained from commercial sources. The Craig polyesters were kindly supplied by Dr. B. M. Craig and were prepared by reaction of adipic acid with polyethylene glycol (Carbowax 400) and 1% diglycerol as plasticizer and succinic or phthalic acid with ethylene glycol (10). The succinate polyester (SEG) was a solid having m.p. 96–100° C, whereas the adipic (APEG) and phthalate (PEG) polyester were viscous liquids.

Commercial samples of liquid terpenes and oxygenated derivatives were purified by fractional distillation and for critical work by GLC, using the Carbowax 400 or APEG polyester columns. Collection of the samples was carried out with the detector current reduced from 250 ma to 100 ma or completely turned off. The refractive indices of the samples thus obtained are shown in Table I.

TABLE I  
Refractive indices of terpenoid compounds

	Boiling point (°C)	$n_D^{20}$	
		Purified by distillation	Purified by GLC
$\alpha$ -Pinene	155–156	} 1.4684	1.4671
$\beta$ -Pinene	—		1.4793
$\alpha$ -Phellandrene	175–176	1.4764	1.4730
$\alpha$ -Terpinene	173.5–174.5	—	1.4780
<i>d</i> -Limonene	176–177	1.4731	1.4722
1,8-Cineole	174.5	1.4583	1.4578
Fenchone	192–193	1.4646	—
Citronellal	205–206	1.4527	—
$\alpha$ -Terpineol	219.8	1.4829	1.4830
Citronellol	222	1.4568	1.4560
Bornyl acetate	223–224	1.4643	—

Eudesmol, m.p. 82–84° C,  $[\alpha]_D +32^\circ$ , was obtained by crystallization and sublimation of a commercial sample (Plaimar Ltd., Perth, Australia) which was obtained from

*Callitropsis araucarioides* Compt. Occidentalol, m.p. 95–96° C,  $[\alpha]_D +363^\circ$ , was obtained from the neutral extractives of the heartwood of *Thuja occidentalis* L. as described by Nakatsuka and Hirose (11).

The mixture of *p*-menthadienes obtained by dehydration of  $\alpha$ -terpineol (9) was prepared by refluxing oxalic acid (120 g) in water (240 ml) with  $\alpha$ -terpineol (40 g) for 6 hours. The mixture was steam-distilled, the distillate extracted with ether, and the ethereal solution was dried over calcium chloride. The mixture of terpene hydrocarbons was used as such after the solvent had been evaporated.

#### *Packing of Columns*

The liquid phase (2.5 g) was dissolved in a volume of low-boiling solvent (50 ml chloroform) which is equal to that of the solid support. This solution was placed into a high, narrow beaker or cylinder and the solid support (12.5 g) was added with stirring. Stirring was continued for 3 to 5 minutes after addition and the mixture was spread on a shallow stainless steel tray. After the solvent had evaporated, the residual material was dried *in vacuo* at 60–70° C for about 1 hour. The dried material was poured in small aliquots with tapping into 6-mm inner diameter copper tubing of 6-foot length, taking note of the weight of mixture added. The column was closed on each end with a small glass wool plug and coiled into a 5- to 7-in. diameter coil. It was allowed to equilibrate at the desired temperature and flow rate of helium gas (0.5 to 1.5 hours). Injection of samples was begun when the recorder maintained a steady base line.

When polyethylene (obtained by cutting polyethylene reagent bottles into small pieces) was used as liquid phase, it was dissolved by refluxing in a minimum volume of petrol (b.p. 154–202° C). The solid support was added to the hot solution as above and the drying time was extended to 5 hours.

## RESULTS AND DISCUSSION

#### *Initial Experiments*

Optimum conditions for each type of column were obtained by trial and error. Columns having Celite 545 as solid support were found to require higher temperatures and inlet pressures than those with C-22 firebrick, but the latter caused decomposition of some compounds at higher temperatures, especially aldehydes and tertiary alcohols. Liberti and Cartoni (4) report that Stermachol, a material similar to C-22 firebrick, is not suitable for terpene alcohols, and used inactivated Celite instead. A more suitable solid support was found in Chromosorb W, which combined the advantages of firebrick without causing decomposition. This support was, therefore, used in all subsequent experiments.

For preparative work a high ratio of liquid phase to solid support is desirable (8, 12), but in the present study the degree of separation deteriorated when 20% or more liquid phase was used. A ratio of 1:6 was found to give consistently good results.

Polyethylene, Apiezon M, polystyrene, and a polyamide (Zytel) gave inferior results and these were not further studied. Solid compounds, such as the SEG polyester (m.p. 96–100° C), gave poor separations below their melting or softening point.

#### *Effect of Physical Factors*

The effect of temperature on the degree of separation of the mixture of *p*-menthadienes on the Carbowax column is shown in Fig. 1. Contrary to the report of Bernhard (1), the effect was found to be considerable. Keulemans (8), from theoretical considerations, also considers the effect to be important. The separation at 80° C, the lowest feasible tem-

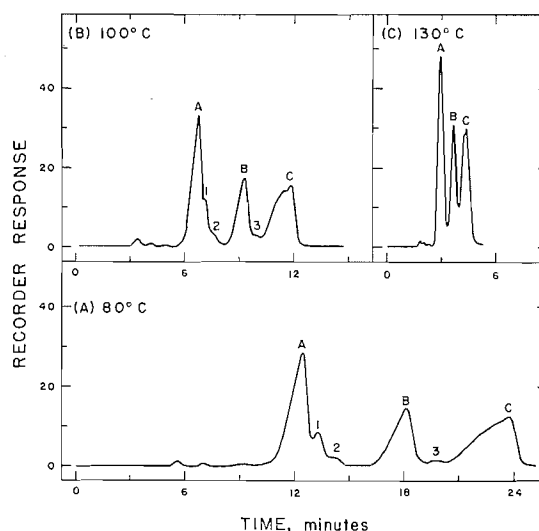


FIG. 1. Separation of the mixture of *p*-menthadienes on the Carbowax column at (A) 80° C and 43.5 ml helium per minute, (B) 100° C and 42.8 ml helium per minute, (C) 130° C and 41.9 ml helium per minute.

perature, was by far the best (Fig. 1(A)), even though the curves are skewed. From a preparative point of view skewing of curves is not detrimental, but is less satisfactory for quantitative calculation. The collection of fractions at the lowest possible temperature has the additional advantage of minimizing the danger of thermal decomposition or rearrangements.

Changes in the flow rate of the carrier gas over the practical range of about 10 to 400 ml (8) did not effect the degree of separation noticeably, which is in agreement with the considerations of Keulemans (8) and the findings of Bernhard (1). Thus, the flow rate was adjusted to give optimum separations at as low a temperature as possible.

The effect of sample size on the retention time of each component was found to be considerable. Table II shows the retention times obtained for *d*-limonene on various columns when samples of 1.5 to 50  $\mu$ l were injected at optimum temperatures and flow rates. From these results it is evident that an increase in sample size above 1 to 3  $\mu$ l

TABLE II  
Effect of sample size on the retention time (in minutes) of *d*-limonene

Column (liquid phase)	Temp. (°C)	Flow rate (ml He/min)	Sample size ( $\mu$ l)							Initial emergence of peak (1.5-50 $\mu$ l)
			1.5	3	5	10	20	40	50	
Silicone (washed)	130	50.5	5.4	5.5	5.6	6.05	6.7*	7.4*	7.8†	4.6-5.0
Silicone (washed)	Duplicate run		5.5	5.6	5.7	6.1	6.8*	7.5*	7.8†	4.7-5.1
Squalene	130	74.2	15.5	15.5	15.6	16.3	17.2*	18.2†	19.0†	14.0-14.3
Carbowax 400	130	46.8	2.85	2.9	2.9	3.0*	3.4*	3.9†	—	2.4-2.5
Polyphenyl ether	130	53.6	6.4	6.55	6.7	6.9*	7.55†	8.1†	—	5.2-5.6
SEG polyester	100	25.0	4.75	4.95	5.35	6.3*	8.2†	—	—	3.6-4.0
APEG polyester	130	41.6	3.95	4.0	4.1	4.3	4.65*	5.25*	5.4†	3.35-3.45
PEG polyester	100	43.5	5.1	5.2	5.5	6.0	6.9*	8.2†	9.0†	3.85-4.25
Diglycerol	80	20.0	2.6	2.75*	3.8†	—	—	—	—	1.2-1.8

\*Skewing clearly visible. †Excessive skewing of peak.

produces considerable delays in the emergence of a peak maximum, and this is accompanied by broadening of the peaks. This is in agreement with the findings of Bens and McBride (13), those obtained by Porter *et al.* (14) with gaseous samples, and those obtained by Bethea and Smutz (15) with aliphatic alcohols on a butyl phthalate column. The latter authors report the optimum sample size for 6-mm diameter columns to be from 4 to 12  $\mu\text{l}$  and detected overloading above 20  $\mu\text{l}$ . Thomas (16) found the optimum for liquid samples to be 30–70  $\mu\text{l}$ . Table II shows that different types of columns have different capacities, silicone and APEG polyester allowing samples of up to 40  $\mu\text{l}$  size, although skewing became noticeable with 10–20  $\mu\text{l}$  samples. Diglycerol has too low a capacity to be attractive for preparative work, but the other liquid phases may be useful since they have a fair capacity and different separation properties.

The fact that larger samples cause a considerable delay of peak maxima has important consequences for both analytical and preparative work. In the former, an unknown peak is often identified by comparing its retention time with those of known compounds run under identical conditions (8). This is only permissible if the sample size of the unknown and known compounds are nearly the same. The technique of adding the known compound to the unknown (8) may be preferred, but actual isolation and comparison of physical constants (e.g., the infrared spectrum) would seem to be a more reliable means of identification. Another alternative is to make use of the fact that the initial emergence time of a peak remains practically constant with different sample sizes up to where the column becomes badly overloaded (14, Table II). However, this can be used only when all peaks are completely separated from one another.

From a preparative point of view the delay of the peak maximum and broadening of the peak caused by a larger sample is not detrimental, provided skewing or broadening does not cause an overlap with neighboring peaks. Even then, an overloaded column may still produce pure fractions if center cuts are collected. The use of columns having larger diameter (12, 17, 18, 19) did not give the same degree of separation as was obtained on the corresponding 6-mm columns when larger samples (50  $\mu\text{l}$  or more) were used. Also, because of the higher flow rates required in larger diameter columns the consumption of helium gas was found to be considerable and it was found more advantageous to carry out repeated runs of 25 to 50  $\mu\text{l}$  on the 6-mm diameter columns. Since most columns required only 15–30 minutes for each run, the collection of 0.1 to 0.5 g of a desired fraction was possible within half a day. When such fractions were collected with a normal detector current of 200 to 250 ma, these were yellowish and on rechromatographing the material, typical decomposition peaks were recorded besides the normal symmetrical peaks for the pure compounds. This difficulty was overcome in most instances by using a detector current of 100 ma, which was sufficient for samples of 20  $\mu\text{l}$  or more. Only in exceptional cases, e.g.  $\alpha$ -pinene or tertiary alcohols, was it necessary to carry out preparative runs without the detector, i.e. fractions were collected at times determined previously in a run of the same sample size with the detector current on. A possible means of overcoming the necessity of "blind" runs may be to construct a bypass system, similar to those used with detectors other than the thermal conductivity type.

#### *Separation Obtained with Various Liquid Phases*

Terpene hydrocarbons were separated most successfully on columns containing squalene, APEG polyester, and polyphenyl ether, separations being in the same order as those obtained on Carbowax 400 and superior to those obtained on silicone (see Table III). Each column appears to offer slight advantages for one or another compound, a finding which was especially apparent in runs with the complex *p*-menthadiene mixture

TABLE III  
Relative retention times (*d*-limonene = 1) of terpene hydrocarbons

Column (liquid phase)	Silicone	Squalene	Carbowax	Polyphenyl ether	APEG polyester
Temperature (° C)	100	100	100	100	100
Flow rate (ml He/min)	50.5	74.2	42.8	92.4	26.3
<i>d</i> -Limonene (retention time in minutes)	16.75	15.4	8.0	13.2	15.6
$\alpha$ -Pinene	0.57	0.52	0.40	0.39	0.39
$\beta$ -Pinene	0.72	0.69	0.59	0.59	0.61
$\alpha$ -Phellandrene	0.84	0.83	0.79	0.81	0.82
$\alpha$ -Terpinene	0.92	0.88	0.825	0.88	0.88

(Figs. 1 and 2). The squalene column is unstable at 130° C for prolonged periods of time, and an efficient aliphatic polyunsaturated liquid phase which is stable at 130° C or higher is still lacking.

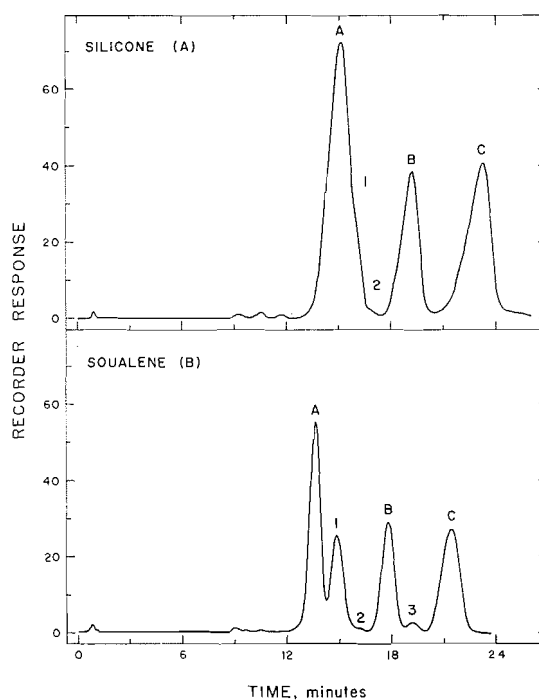


FIG. 2. Separation of the mixture of *p*-menthadienes on (A) silicone (at 100° C and 50.5 ml helium per minute), (B) squalene (at 100° C and 74.2 ml helium per minute).

Resolution of the mixture of *p*-menthadienes on the silicone column (cf. Fig. 2(A)) shows three major peaks A, B, and C, with an indication of further constituents at 1 and 2. The squalene (cf. Fig. 2(B)) and the Carbowax columns (cf. Fig. 1) clearly showed three additional components, 1, 2, and 3, and on the latter column peak C appears to be composed of two compounds in about equal amounts. A very similar separation was obtained with the APEG and SEG polyester columns, whereas the polyphenyl ether column gave the same resolution except that C appeared as a single peak. The best resolution of A, which corresponds to  $\alpha$ -terpinene, was obtained on the squalene column, separation from impurity 1 being complete. From these results it follows that the com-

plex mixture of *p*-menthadienes obtained by dehydration of  $\alpha$ -terpineol cannot be resolved by one column alone, but requires successive fractionation on the squalene and polyester columns. For analytical purposes longer columns or the very efficient Golay columns (20, 21) may have to be used. Peak C presents a problem and its composition, as well as the chemical identification of the other components will be the subject of another communication.

TABLE IV  
Relative retention times (*d*-limonene = 1) of various monoterpenoid compounds

Column (liquid phase)	Silicone	Squalene	Carbo- wax	Poly- phenyl ether	Polyesters		Digly- cerol
					APEG	SEG	
Temperature ( $^{\circ}$ C)	130	130	130	130	130	100	100
Flow rate (ml He/min)	30.0	74.2	42.0	53.6	41.6	26.3	20.0
Inlet pressure (mm Hg)	1531	2029	1531	1531	1531	1531	1014
Retention time of <i>d</i> -limonene	8.3	15.5	3.15	6.0	3.8	2.0	1.5
Specific retention volume $V_g$	19.1	27.2	19.1	23.0	15.9	9.0	8.9
1,8-Cineole (b.p. 174 $^{\circ}$ )	1.1	0.95	1.25	1.2	1.25	1.05	1.5
Fenchone (b.p. 192 $^{\circ}$ )	1.4	—	3.0	2.2	2.9	3.0	3.2
Citronellal (b.p. 205 $^{\circ}$ )	2.0	—	3.8	3.0	3.65	4.2	3.25
Camphor (sublimes)	1.95	—	5.15	3.7	5.1	5.6	6.4
$\alpha$ -Terpineol (b.p. 220 $^{\circ}$ )	2.45	—	11.6	4.7	10.95	11.5	6.6
Citronellol (b.p. 222 $^{\circ}$ )	3.1	—	20.2	4.45	14.6	13.3	6.7
Bornyl acetate (b.p. 223 $^{\circ}$ )	3.85	—	6.2	5.5	5.95	6.2	4.8

Table IV shows the relative retention volumes with respect to *d*-limonene of some oxygenated derivatives. The best degree of separation of these compounds was obtained on the Carbowax and APEG polyester column, and that of the former is compared in Fig. 3 with the separation obtained on silicone (Fig. 3(A)). Since Carbowax cannot be

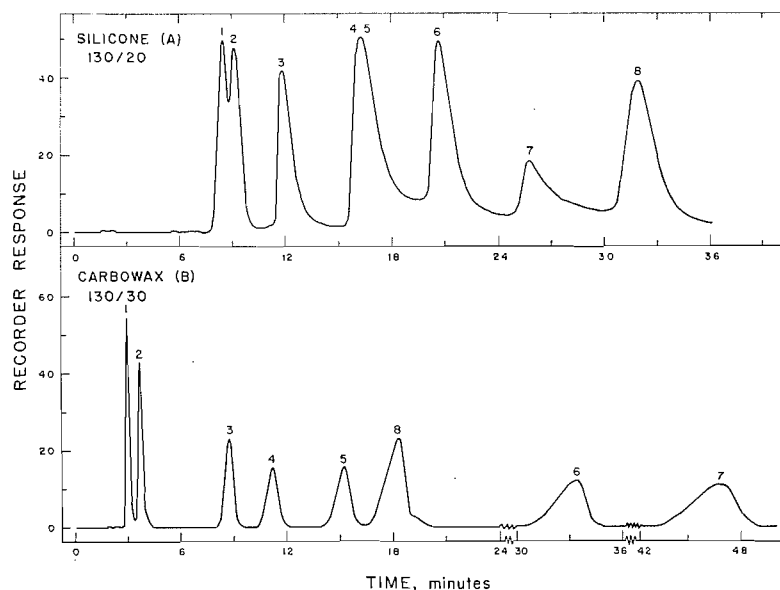


FIG. 3. Separation of terpenoid compounds on (A) silicone (at 130 $^{\circ}$  C and 30.0 ml helium per minute), (B) Carbowax (at 130 $^{\circ}$  C and 42.0 ml helium per minute).

1, limonene; 2, 1,8-cineole; 3, fenchone; 4, citronellal; 5, camphor; 6,  $\alpha$ -terpineol; 7, citronellol; 8, bornyl acetate.

used above 100° to 120° C for long periods (8), the use of APEG polyester is to be preferred, especially so, since the latter has also a higher capacity (cf. Table II) and can be used for high boiling sesquiterpene alcohols and monophenols (see below) as well. All columns, except squalene, eluted *d*-limonene (b.p. 177° C) ahead of 1,8-cineole (b.p. 174° C) and all, except silicone and polyphenyl ether, eluted the higher boiling bornyl acetate ahead of  $\alpha$ -terpineol and citronellol. Bornyl acetate was eluted even ahead of camphor on the diglycerol column. Thus the use of different liquid phases can be used to differentiate between different functional groups (22). The sequence of separations on polyphenyl ether as compared with that obtained on the polyesters is noteworthy. From the present findings it is concluded that no single column will satisfy all requirements.

Silicone grease and the Craig polyesters (10) are known to be sufficiently stable at 160 to 220° C to allow the chromatography of compounds with higher boiling points than  $\alpha$ -terpineol. In addition, the polyphenyl ether was found to be suitable for runs at higher temperatures, and Table V shows the degree of separation of the closely related

TABLE V  
Relative retention times ( $\alpha$ -terpineol = 1) of sesquiterpene alcohols

Column (liquid phase)	Silicone	Polyphenyl ether	Polyesters		
			APEG	SEG	PEG
Temperature (° C)	160	160	190	190	190
Flow rate (ml He/min)	99	120	41.6	39.4	62.5
Inlet pressure (mm Hg)	2546	2546	1531	2029	2029
Retention time of $\alpha$ -terpineol	3.1	6.6	5.5	5.8	3.0
Occidentalol	4.9	4.6	3.4	3.0	3.0
Eudesmol a	6.5*	7.0*	5.25	5.1	4.5
Eudesmol b	—	—	5.4	5.3	4.7

\*Broad, unresolved peak.

sesquiterpene alcohols, occidentalol ( $C_{15}H_{24}O$ ) and eudesmol ( $C_{15}H_{26}O$ ), as compared with  $\alpha$ -terpineol obtained with these columns. Occidentalol differs structurally from eudesmol only by having one more double bond, yet it is completely separated from the latter even on the silicone column. This may, in part at least, be due to steric differences. The superior efficiency of the polyester columns is evident in that eudesmol is resolved partially into the  $\alpha$ - and  $\beta$ -isomers.

Since essential oils sometimes contain phenolic compounds, the degree of separation of the monophenols, anethole, eugenol, and isoeugenol, was tested on the APEG polyester and the polyphenyl ether columns. The former, at 190° C and 83.5 ml helium per minute, gave retention times of 5.3, 14.7, and 25.4 minutes, whereas the latter, at 220° C and 70.5 ml helium per minute, gave 4.3, 6.45, and 10.4 minutes respectively. Both columns gave even peaks without any sign of decomposition taking place. The separation of other types of phenols has been described by Janák *et al.* (23, 24).

#### CONCLUSIONS

1. Monoterpene hydrocarbons are best separated on columns using squalene, Carbowax 400, Craig polyesters, and polyphenyl ether as liquid phase at temperatures of 130° C and lower. At higher temperatures the polyester and polyphenyl ether columns give best results, separating oxygenated terpenes, sesquiterpene alcohols, and monophenols satisfactorily. The adipate polyethylene glycol (APEG) polyester column is most suited for all compounds tested.

2. The effect of temperature and sample size on the degree of separation is considerable, the lowest possible temperature giving best results from a qualitative and preparative point of view. With 6-mm diameter columns sample sizes above about 3  $\mu$ l cause considerable delays in the retention time of peak maxima as well as a broadening of peaks. The initial emergence of a peak is practically constant up to sample sizes which cause overloading of the columns. For qualitative work actual isolation of each component and determination of its physical properties is to be preferred over comparison of the retention time (or volume) with those of known compounds.

3. Chromosorb W, a calcined diatomaceous earth, was found to be a better solid support for the separation of terpenoid compounds than either washed Celite 545 or C-22 firebrick.

4. In preparative work with instruments using a thermal conductivity cell as detector, pure samples cannot be obtained with normal detector currents, and collection of thermally labile terpenes must be carried out in "blind" runs.

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