GAS–LIQUID CHROMATOGRAPHY OF TERPENES

PART III. THE USE OF OLEIC ACID ESTERS AS LIQUID PHASES[1]

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ABSTRACT

The separation of several cyclic terpene hydrocarbons and some oxygenated derivatives was studied on a variety of triglyceride and dioleate ester columns. Use of rapeseed oil, partially hydrogenated rapeseed oil, olive oil, triolein, tristearin, methyl oleate, and potassium oleate as liquid phases led to the conclusion that the presence of an esterified mono-unsaturated long-chain acid is a desirable constituent of the liquid phase. Dioleate esters of 1,3-propanediol, 1,4-butanediol, 1,6-hexanediol, diethylene glycol, and polyethylene glycol showed useful differences in the degree of separation of both terpene hydrocarbons and oxygenated derivatives. Ether linkages, as in the di- and poly-ethylene glycol ester, also were associated with favorable separations. When the liquid phase contained free hydroxyl groups, the degree of separation of hydrocarbons and ketones was in general less favorable. The spacing of the ester groups was found to have some effect on retention data. The separation of such critical pairs of isomers as tricyclene and α-pinene, α-fenchene and camphene, and also geometrical isomers of oxygenated derivatives is facilitated by use of several of these liquid phases.

During an examination of the essential oil of the leaves of Eastern white cedar (*Thuja occidentalis* L.) by means of gas–liquid chromatography (GLC) (1), the peak corresponding in retention time to camphene was isolated and its infrared spectrum was found to correspond to that of synthetic camphene. Yet the sample failed to crystallize on seeding. Further examination on the rapeseed oil column (2) indicated a second component, presumably α-fenchene (3), to be present (partially resolved peak). There was no indication of this impurity on either the 6-ft adipate polyester (APEG) column or polyphenyl ether columns (2, 4). Other such unresolved or partially resolved pairs of isomeric terpenes are frequently encountered, e.g. tricyclene and α-pinene (3), terpinolene and $\Delta^{2,4(8)}$-*p*-menthadiene (2), or the many geometrical and positional isomers of oxygenated terpenes. Thus, for either qualitative or quantitative analyses by GLC of mixtures of terpenes and especially essential oils, improved separations over those previously obtained seem imperative. Slight improvements were obtained with the apparatus used in this study by some modification of the injector (see experimental part) and by using less than 5 μl amounts, but this did not lead to the desired results. Bernhard (5) has pointed out that the most useful experimental parameter is the choice of stationary liquid phase, and has investigated a large variety of compounds for use in the separation of lemon oil constituents. This aspect was further investigated in this study.

The use of polyesters as liquid phases in GLC separation has been studied fairly extensively (3–10) and the results obtained with adipate polyesters (4, 5) were a considerable improvement over older results. However, columns with these liquid phases failed to separate several of such critical pairs of isomers. In the previous paper of this series (2), it was shown that vegetable oils, such as rapeseed and olive oil, were very suitable for the separation of monocyclic terpene hydrocarbons and it was suggested that triolein may be useful as a liquid phase. This has now been found to be so, and it could also be shown that these triglyceride columns give useful retention data for oxygenated monocyclic terpenes.

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During investigations on GLC separations of methyl esters of fatty acids in this laboratory, Craig (11) has observed that the spacing of the ester group along a polyester chain can have significant effects on the degree of separation. Thus, it is conceivable that the close proximity of three ester groups in the triglycerides is not necessarily the most favorable arrangement for the separation of terpenoid compounds. Therefore, a number of dioleate esters were prepared in which the ester groups were spaced progressively further from one another, viz. esters of 1,3-propanediol, 1,4-butanediol, 1,6-hexanediol, diethylene glycol, and polyethylene glycol. Oleate esters were chosen because the data obtained with the triglycerides suggested that oleic acid was the most desirable acidic component in these polyesters. In addition, the polyester of azelaic acid with diethylene glycol was tested to obtain further data on the spacing of the ester groups.

EXPERIMENTAL

Apparatus

A Beckman GC-2 chromatograph equipped with a thermal conductivity cell was modified by placing the injector as close as possible to the head of the column. The temperature of the injector was kept 20–25° C higher than that of the column and the detector cell. All experiments were carried out with an inlet pressure of 55 p.s.i. (3.74 atm) and the column outlet at atmospheric pressure (0.93 ± 0.02 atm). The flow rates were measured with a moving soap bubble at room temperature. The chart speed of the 1-mv recorder was 10 in. per hour, and samples were injected with a hypodermic syringe.

Preparation of Columns

All liquid phases were applied to the solid support Chromosorb W (Johns-Manville) in the ratio of 6 to 1 by weight. The liquid phase (2.0 g) was dissolved in chloroform (45 ml) and to the solution was added, with stirring, the solid support (12.0 g). The resulting thick slurry was stirred for 5–10 minutes, transferred to a shallow tray and stirred intermittently until the slurry became a dry powder. The material was then dried *in vacuo* at 50–60° C for 1 hour and packed with constant tapping into 6 ft by $\frac{1}{4}$ in. O.D. copper columns. The weights of liquid phase and solid support for each column are shown in Table I. All columns were used for a number of runs before critical measurements were taken.

Synthesis of Oleic Acid Esters

The technique described by Youngs *et al.* (12, 13) was followed throughout. In a typical example oleic acid (6.2 g, 22 mmoles, commercial 95%) was dissolved in dry petrol (b.p. 60–70° C) (65 ml) and to this was added phosphorus pentachloride (5.0 g, 24 mmoles). The mixture was heated under reflux with exclusion of moisture for 1 hour, then cooled and quickly washed twice with ice water (13). After the petrol solution was dried over anhydrous sodium sulphate, the solvent was evaporated. To the residual oleyl chloride was added 1,3-propanediol (0.76 g, 10 mmoles) and the mixture was heated on a rotary evaporator at 50–60° C for 6 hours, the initial vacuum being gradually increased to 10 mm Hg to remove the hydrochloric acid liberated. Any attempts to remove unreacted oleic acid by washing a solution of the crude reaction product with aqueous alkali resulted in very stable emulsions. Therefore, the residual reaction product was chromatographed on a 3×50 cm column of silicic acid, the ester being eluted with 100-ml aliquots of petrol:chloroform (1:1 v/v). The first three fractions had an infrared spectrum expected for the diester but acidic impurities could be detected, especially in

TABLE I

Physical constants of the 6 ft×¼ in. columns

Liquid phase	Abbrevn.	Weight with solid support (g)	80° C			110° C			Retention time of air peak	
			Flow rate*	*t* limonene†		Flow rate*	*t* camphor†			
				(*a*)	(*b*)		(*a*)	(*b*)	80° C	110° C
Rapeseed oil	—	10.0	113	31.1	32.3	94	37.2	39.0	0.4	0.4
Olive oil	—	9.5	103	29.3	30.5	85	32.8	35.0	0.4	0.4
Triolein	—	10.3	125	28.3	29.5	109	29.4	31.2	0.3	0.3
Hydrogenated rapeseed oil I	HRO I	11.1	110	33.1	34.5	100	40.3	42.9	0.4	0.5
Hydrogenated rapeseed oil II	HRO II	10.5	110	35.4	37.5	100	42.3	45.8	0.3	0.3
Tristearin	—	10.1	120	36.0	39.1	95	28.9	32.5	0.3	0.3
Methyl oleate	—	10.5	94	71.5	75.0	—	—	—	0.5	—
Potassium oleate	—	10.9	78	9.9	10.9	—	—	—	0.3	—
1,3-Propanediol dioleate	PDDO	10.0	81	64.1	66.5	66	51.9	56.6	0.4	0.4
1,4-Butanediol dioleate	BDDO	10.5	127	32.9	33.9	113	32.1	35.0	0.3	0.3
1,6-Hexanediol dioleate	HDDO	10.4	86	51.8	53.3	75	45.0	47.5	0.3	0.3
Diethylene glycol dioleate	DEGDO	10.8	88	23.5	24.5	80	27.5	28.8	0.4	0.4
Polyethylene glycol dioleate	PEGDO	10.6	113	25.3	26.3	100	35.1	37.0	0.3	0.4
Polyethylene glycol monooleate	PEGMO	11.0	100	32.4	34.1	85	39.0	41.5	0.4	0.4
Polyoxyethylene sorbitan trioleate	Tween 85	10.2	120	17.4	19.0	100	21.8	24.5	0.2	0.3
Polyethylene glycol polyadipate	APEG	12.5	100	10.2	11.3	95	30.7	32.5	0.3	0.3
Diethylene glycol polyazelate	AzDEG	11.6	103	12.0	13.0	104	24.3	24.0	0.2	0.3
Polyethylene glycol	PEG 1540	11.2	89	13.6	14.6	67	30.8	32.7	0.4	0.4
m-bis(*m*-Phenoxyphenoxy)-benzene	PPE	11.5	150	17.9	19.2	150	28.3	30.5	0.2	0.2

*In ml helium per minute.
†Retention time: (*a*) measured to peak base; (*b*) measured to peak maximum.

the third fraction. The material was combined by dissolving in chloroform (10 ml), solid anhydrous potassium carbonate (1.0 g) was added, and the mixture was stirred for 10–15 minutes. It was then rechromatographed on silicic acid to give the crude diester (3.6 g, 60% of theory). The infrared spectrum of the eluted material showed no longer the presence of free acid, but elementary analysis gave low values for the percentage carbon (up to 0.8%). On distilling the crude diester *in vacuo* (b.p. 195° C at 0.2 mm, air bath) the colorless diester was obtained practically pure. Found: C, 76.90%; H, 11.98%; neutral equivalent, 3; saponification equivalent, 297. Calculated for $C_{39}H_{76}O_4$: C, 76.91%; H, 12.58%; saponification equivalent, 304.50.

Where the yield of the diester was poor, a substantial amount of the corresponding monoester was recovered from the silicic acid column by eluting first any unreacted oleic acid with chloroform and then eluting further with chloroform – 2-butanone (1:1 v/v). The crude esters were all light to medium yellow in color and were obtained colorless only after distillation. Polyethylene glycol mono- and di-oleate failed to distill at a pressure of 0.1 mm Hg, and any residual oleic acid which may have been present after chromatography was converted to methyl oleate by treatment with an ethereal solution of diazomethane.

Materials

Triolein, tristearin, and the partially hydrogenated rapeseed oils were kindly donated by Dr. C. G. Youngs. Two different samples of partially hydrogenated rapeseed oil were used: I (HRO I), having a content of 14% saturated fatty acids, and II (HRO II) with 50% saturated acids. The adipate polyester of polyethylene glycol (APEG) and azelate polyester of diethylene glycol (AzDEG) were a gift kindly made by Dr. B. M. Craig (6). The polyphenyl ether [*m*-bis(*m*-phenoxyphenoxy)-benzene], "Tween 85" (polyoxyethylene sorbitan trioleate), and polyethylene glycol (Carbowax 1540) were commercial products.

The terpenes used in this study were either commercial samples or those previously prepared (2). The mixture of *trans*- and *cis*-dihydro-α-terpineol was prepared by hydrogenating α-terpineol with platinum oxide as catalyst (14). In addition a commercial sample of the essential oil of cedar leaf (*Thuja occidentalis* L.) was used.

PRESENTATION OF DATA

In 1958, Ambrose *et al.* (15) and Johnson and Stross (16) put forward proposals for a unified way of presenting GLC data. It was pointed out that reproduction of the actual recorder chart gave the least amount of information in a maximum amount of space. Tables of relative retention times with respect to a standard, or standards, were to be preferred, but far more useful presentation would result from calculation of retention volumes (corrected or uncorrected) and(or) partition coefficients. However, all such calculated data are obtained from the basic equation $V_R = F_c t'$ (15) where V_R is the retention volume, F_c the flow rate of carrier gas at column temperature, and t' the time of emergence of the peak maximum. Now it was shown (4, 17, 18, 19) that the position of the peak maximum of a given compound varies considerably with the size of the injected sample (over the practical range of about 0.1 to 50 μl used with the type of apparatus employed in this study). Also, the retention time may be longer if the compound is preceded by a much larger quantity of another compound (20). Thus, unless all measurements are carried out with identical sample sizes, considerable errors will

result in all of the calculated data. The use of equal sample sizes is not only time consuming but is a practical impossibility in essential oils and many mixtures of synthetic terpenes. Thus, for practical purposes at least, comparison of the actual recorder tracing (chromatogram) will remain the most reliable means of comparing column efficiencies. Next best, possibly, is the tabulation of relative retention times (with respect to a standard compound) *as measured from the point of injection to the initial emergence* of the peaks under consideration (2). The latter procedure is also subject to small errors when peaks are not completely separated. Further objections may be raised since the initial emergence is dependent upon detector sensitivity and thus small fluctuations in the base line may obscure detection of the initial emergence of a peak to some extent. These difficulties can be overcome, in part at least, by drawing the tangent to the peak and using the intercept at the base line as reference point. However, this is also subject to small variations with different sample sizes and the initial emergence point is to be preferred. Therefore, the data obtained in the present study was tabulated as relative retention times with respect to a standard and as measured to the initial emergence of each peak.

Retention times, or relative retention times, give no information on the peak width. Thus, whilst the relative retention times of two compounds may be the same on different columns, the actual separation achieved will only become apparent on comparing the recorder charts. Also, in separations involving essential oils different types of organic compounds are involved and the relative peak width of, for example, the hydrocarbons may be different from those of the ketones, alcohols, etc. Separation factors or functions have been put forward (21, 22, 23) to overcome this difficulty, and Jones and Kieselbach (24) have proposed the use of the ratio of retention time to peak width (relative peak sharpness) as a measure of the efficiency of a column. The latter quantity appears to offer advantages since it is a direct measure and not a theoretical concept relating to an idealized mode of operation. However, if the retention time is measured at the peak maximum, the error discussed above will once again make comparison between peaks of different sample sizes unreliable. Also, peak width varies with sample size and a smaller value for the relative peak sharpness will be obtained with larger sample size. These same observations apply to the use of retention indices as developed by Kováts (25). Thus, the visual examination of the charts still remains the most reliable means of determining column efficiency when peaks of varying sample size are involved. However, some measure of the degree of separation can be obtained by comparing the retention time of the point of initial emergence of a peak with that of the peak maximum. This is shown in Table I for all the columns studied for limonene (at 80° C) and for camphor (at 110° C). Flow rates, retention times of air, and the total weight of the liquid phase and solid support are also listed to allow other calculations, if desired.

RESULTS AND DISCUSSION

The relative retention times of monoterpene hydrocarbons, 1,8-cineole and *p*-cymene with respect to limonene as obtained on the rapeseed oil, tristearin, methyl oleate, and potassium oleate columns are compared in Table II with those obtained on a polyethylene glycol (PEG 1540) column. Only insignificant differences in the relative retention times were obtained with the rapeseed oil, olive oil, trioleate, and methyl oleate columns and only the data for the former are shown. Visual comparison showed a slightly better degree of separation for the triolein column, whilst that obtained with olive oil was

TABLE II

Relative retention times (limonene = 1.00) of cyclic terpene hydrocarbons on 6 ft × ¼ in. triglyceride columns at 80° C

l limonene† (minutes)	31.1	33.1	35.4	36.0	9.9	13.6
Liquid phase	Rapeseed oil	HRO I*	HRO II*	Tristearin	Potassium oleate	PEG* 1540
1. Tricyclene	0.35	0.39	0.39	0.37	0.40	0.32
2. α-Pinene	0.39	0.425	0.43	0.41	0.44	0.325
3. Unknown‡	0.44	0.47	0.50	0.48	0.505	0.44
4. Camphene	0.465	0.49				
5. β-Pinene	0.56	0.59	0.62	0.61	0.625	0.56
6. Unknown§	0.63	0.65	0.64			0.59
7. α-Phellandrene	0.80	0.82	0.80	0.80	0.78	0.775
8. α-Terpinene	0.87	0.89	0.89	0.87	0.81	0.88
9. Limonene	1.00	1.00	1.00	1.00	1.00	1.00
10. γ-Terpinene	1.30	1.28	1.29	1.27	1.22	1.30
11. Terpinolene	1.56	1.45	1.56	1.51	1.49	1.65
12. $\Delta^{2,4(8)}$-*p*-Menthadiene	1.64	1.50				
13. 1,8-Cineole	0.94	0.94	0.92	1.00	1.00	1.125
14. *p*-Cymene	1.06	1.08	1.07	1.01	1.01	1.50
15. Fenchone	1.82	1.52	—	—	—	3.40

*Abbreviations as used in Table I.
†Retention time of limonene as measured from injection to initial emergence of peak.
‡From cedar leaf oil, possibly α-fenchene.
§From cedar leaf oil, possibly myrcene.

slightly worse than that for rapeseed oil. Methyl oleate did not only give very high retention times (see Table I), but was found to bleed at higher temperatures. With the more saturated triglycerides a decrease in efficiency was noted with increasing concentrations of saturated esters. Quite different results were obtained with potassium oleate; the retention times were very low and the resolution was rather poor. Since more unsaturated vegetable oils, such as linseed and sunflower oils, gave also poorer results than rapeseed oil (2), there is little doubt that esterified oleic acid is the most desirable component in this type of liquid phase.

Pure oleate esters will not oxidize as rapidly as when they are contaminated with more unsaturated esters. However, this advantage of synthetic triolein over the vegetable oils may be offset by the difficulties encountered in preparing pure triolein. Deterioration of the vegetable oil columns was conveniently detected by a decrease in the separation between 1,8-cineole and limonene; a 6-month-old rapeseed oil column gave even slightly longer retention times for the oxide. No noticeable differences in the separation of isomeric hydrocarbons were detected, unless the deterioration was severe. This behavior of the oleate columns may be due to slow oxidation of the liquid phase resulting in a gradual increase in polarity and thus approaching the polyether or polyester type of separation. The results obtained with rapeseed oil, triolein, and partially hydrogenated rapeseed oil I (14% saturated fatty acids) show that the purity of the triglycerides with respect to saturation is not critical up to a content of about 10% of saturated acids. Also, small variations in the chain length of the unsaturated moiety (as found in rapeseed oil) do not appear to have a marked effect on the degree of separation of terpene hydrocarbons.

Table III shows the relative retention times of the same terpenes obtained with synthetic dioleates, polyethylene glycol monooleate, "Tween 85", azelate polyester (AzDEG), and adipate polyester (APEG) as liquid phase. 1,3-Propanediol dioleate gave

TABLE III

Relative retention times (limonene = 1.00) of cyclic terpene hydrocarbons at 80° C on 6 ft × $\frac{1}{4}$ in. dioleate columns

t limonene† (minutes)	32.9	51.8	23.5	25.3	32.4	17.4	10.2	12.0
Liquid phase	BDDO*	HDDO*	DEGDO*	PEGDO*	PEGMO*	Tween 85*	APEG polyester*	AzDEG polyester*
1. Tricyclene	0.36	0.37	0.36	0.345	0.35	0.345	0.31	0.30
2. α-Pinene	0.41	0.41	0.40	0.375	0.39	0.37	0.33	
3. Unknown‡	0.46	0.46	0.45	0.45	0.455	0.46	0.46	0.41
4. Camphene	0.48	0.48	0.47	0.47	0.47	0.47	0.47	0.49
5. β-Pinene	0.62	0.61	0.59	0.59	0.59	0.58	0.58	0.56
6. Unknown§	0.68	0.69	0.65	0.65	0.60		0.60	0.625
7. α-Phellandrene	0.82	0.84	0.80	0.79	0.81	0.83	0.80	0.79
8. α-Terpinene	0.88	0.90	0.86	0.855	0.88	0.86	0.83	0.87
9. Limonene	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
10. γ-Terpinene	1.27	1.29	1.25	1.28	1.28	1.28	1.33	1.33
11. Terpinolene	1.54	1.55	1.51	1.59	1.60	1.59	1.60	1.65
12. $\Delta^{2,4(8)}$-*p*-Menthadiene	1.62	1.61	1.58	1.63		1.64	1.63	1.71
13. 1,8-Cineole	1.05	0.98	0.95	1.14	0.99	1.02	1.06	1.21
14. *p*-Cymene	1.12	1.13	1.08	1.25	1.22	1.25	1.54	1.48
15. Fenchone	2.01	1.82	1.83	2.37	2.11	2.10	3.50	3.20

*Abbreviations as used in Table I.
†Initial emergence of peak.
‡From cedar leaf oil, possibly α-fenchene.
§From cedar leaf oil, possibly myrcene.

similar results to those obtained with the unsaturated triglycerides and the relative retention times are therefore not shown. All dioleates gave favorable separation, especially so for the critical pairs of peaks 5 and 6 and peaks 11 and 12. The separation in the α-terpinene to γ-terpinene range were especially good on the di- and poly-ethylene glycol dioleate columns. Noteworthy is also the finding that on most of these columns fenchone was eluted relatively later than on the triglyceride columns. The retention times of 1,8-cineole with respect to limonene varied considerably and no correlation between the degree of separation and the spacing of the two ester groups could be detected. Also, the much shorter retention times obtained on the 1,4-butanediol dioleate (BDDO) column (in part due to the higher flow rate in this column) as compared with those on the propanediol or hexanediol dioleate columns did not result in a markedly different degree of separation, except that 1,8-cineole was eluted later with limonene. The presence of free hydroxyl groups (polyethylene glycol monooleate, polyethylene glycol, and "Tween 85") results in a loss of over-all efficiency. This can also be seen from the data obtained earlier with a polyphenyl ether column (2). Together with the difference in separation obtained on the polyester (APEG and AzDEG), polyphenyl ether (PPE), and polyethylene (PEG 1540) columns these results lead to the conclusion that for the separation of isomeric terpene hydrocarbons, mono-unsaturated hydrocarbon chains and ester groups are favorable, but free hydroxyl groups not. The ether linkage of the polyethers is, in general, favorable, too, and results in a longer retention of 1,8-cineole and *p*-cymene.

For the study of the composition of essential oils, columns which are equally efficient for the separation of oxygenated compounds, including isomeric pairs, are required. The separation of the isomeric menthols on either polyethylene glycol (26) or silicone oil (27) has been reported, and Ofner *et al.* (28) have separated the cis–trans isomers of

aliphatic sesquiterpenoid alcohols on Apiezon. Also, the retention time of the fastest-moving monoterpenoid ketone (usually fenchone) should be such as to allow complete separation of the monoterpene fraction. For this reason the relative retention time of fenchone at 80° C on some of the columns investigated has been included in Tables II and III. The relative retention times with respect to camphor of a number of closely related ketones and alcohols and bornyl acetate as obtained with the above columns at 110° C are shown in Table IV. Though only a relatively small number of oxygenated monoterpenes were studied, the retention data obtained allow conclusions to be drawn as to which column or columns may be suitable for a specific separation. Since the differences obtained with the various triglycerides followed the same pattern as described above, only the results obtained with rapeseed oil and tristearin as liquid phase are shown as typical examples. It is noteworthy that the drop in efficiency in going from the unsaturated to the fully saturated triglyceride was not as marked as in the case of isomeric terpene hydrocarbons. However, this may no longer be true if unsaturated ketones or alcohols are involved. The triglyceride columns tend to separate approximately according to boiling point, showing that these columns are intermediate in polarity as compared with saturated hydrocarbon or silicone and the polyester type of columns.

Very marked differences in relative retention times were obtained on the various dioleate columns. On 1,3-propanedioleate (PDDO) the most remarkable difference is that *trans*-dihydro-α-terpineol and borneol as well as the cis isomer of the former and isoborneol were not separated from one another, whilst on the other oleate columns the cis isomer and borneol would fall together. The relative retention times on the 1,4-butanediol dioleate (BDDO) column were similar to those obtained with rapeseed oil, except that bornyl acetate was eluted more rapidly. This was also so on the polyethylene glycol dioleate (PEGDO) column, only here the separation between *cis*-dihydro-α-terpineol and borneol was superior. The hexanediol dioleate (HDDO) column eluted bornyl acetate more slowly. The most noticeable differences were obtained with diethylene glycol dioleate (DEGDO). Not only are the retention times lower than on the other oleate columns, but the relative retention times of the ketones are noticeably higher. The degree of separation on the polyethylene glycol monooleate column was of the same order as that obtained with tristearin, except that bornyl acetate was eluted ahead of α-terpineol.

Comparison of the retention data obtained on the triglyceride and oleate ester columns with those found on the polyester and polyether columns shows some useful differences. The former columns all gave better resolution of the hydrocarbons. This, however, appears to be at the expense of longer retention times. Regarding the polyester of azelaic acid (AzDEG), also studied in this series (see Tables III and IV), a slight but definite improvement in the separation of hydrocarbons with respect to the adipate polyester polyethylene glycol column was obtained. Since the retention time of limonene was less than half that of most of the oleate ester columns, this liquid phase offers advantages in routine analyses. The separation of oxygenated terpenes on this column was of the same order as that obtained on the adipate polyester column. Previously it was found (2) that succinate polyester was less efficient as liquid phase than adipate polyester as far as separation of terpenes is concerned. Thus, there appears to be a definite advantage in having the ester groups spaced more than four carbon atoms apart. This relationship will, of course, not hold for all types of compounds to be separated, but it confirms the observation made by Craig (11) that the distance of polar groups along the chain of the polymer may have a significant influence on the degree of separation.

TABLE IV

Relative retention times (camphor = 1.00) of oxygenated terpenes at 110° C

t camphor† (minutes)	39.0	30.4	30.8	28.3	30.7	24.3	51.9	32.1	45.0	27.5	35.1
Liquid phase	Rapeseed oil	Tristearin	PEG* 1540	PPE*	APEG*	AzDEG*	PDDO*	BDDO*	HDDO*	DEGDO*	PEGDO*
1. 1,8-Cineole	0.38	0.425	0.27	0.27	0.23	0.23	0.48	0.37	0.395	0.28	0.27
2. Limonene	0.43		0.215	0.23	0.19	0.19		0.39		0.32	0.305
3. Fenchone	0.62	0.61	0.56	0.57	0.52	0.555	0.66	0.62	0.64	0.76	0.59
4. α-Thujone	0.735	0.75	0.655	0.66	0.615	0.655	0.74	0.72	0.76	0.74	0.71
5. Isothujone	0.84	0.83	0.74	0.73	0.74	0.73	0.84	0.82	0.86	0.86	0.78
6. Menthone	1.02	0.84	0.74	0.90	0.75	0.78	1.05	1.02	1.06	0.99	0.96
7. Isomenthone	1.17	0.98	0.88	1.06	0.94	0.95	1.20	1.17	1.14	1.10	1.07
8. Camphor	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
9. *trans*-Dihydro-α-terpineol	1.25	1.40	1.22	0.78	1.09	1.275	1.38	1.33	1.29	0.985	1.20
10. *cis*-Dihydro-α-terpineol	1.40	1.53	1.43	0.90	1.30	1.46	1.51	1.51	1.40	1.13	1.51
11. Borneol	1.41	1.45	2.02	0.93	1.90	1.69	1.34	1.51	1.39	1.13	1.69
12. Isoborneol	1.60	1.63	2.48	1.05	2.24	2.08	1.52	1.71	1.53	1.28	1.93
13. α-Terpineol	1.88	2.04	2.43	1.31	2.45	2.41	2.02	2.13	1.90	1.52	2.17
14. Bornyl acetate	1.89	2.09	1.33	1.56	1.33	1.33	2.21	1.92	2.10	1.53	1.71

*Abbreviations as used in Table I.
†Initial emergence of peak.

The application of the liquid phases found to be most useful in the study of the components of the essential oil of cedar leaves is described in the following communication.

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