Journal of Chromatography, 646 (1993) 307–316
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 277

Retention behaviour of volatile compounds in normal-phase high-performance liquid chromatography on a diol column

Markus Lübke* and Jean-Luc Le Quéré

INRA, Laboratoire de Recherche sur les Arômes, 17 Rue Sully, 21034 Dijon Cédex (France)

Denis Barron

Université Joseph Fourier, UFR de Pharmacie, Laboratoire de Pharmacognosie, 38706 La Tronche Cédex (France)

(First received February 4th, 1993; revised manuscript received April 27th, 1993)

ABSTRACT

Retention data on a diol column for over 300 compounds of the chemical classes usually contained in aroma extracts of plants and foodstuffs are reported. A concept that largely corrects for minor fluctuations of the mobile phase composition and of the flow-rate was used to measure capacity factors. The mobile phase was composed of pentane and diethyl ether. The high volatility of these two solvents makes the method perfectly adaptable to the prefractionation of aroma extracts and the semi-preparative isolation of compounds. Non-polar compounds such as hydrocarbons are not retained on diol. Polar compounds can be readily eluted, with the exception of strong acids and bases.

INTRODUCTION

For over 30 years, the preferred tool of aroma researchers has been capillary gas chromatography (GC). This technique is well adapted to the analysis of volatile compounds. High-performance liquid chromatography (HPLC) certainly cannot compete with GC in terms of separation efficiency or, if so, not within an acceptable analysis time. However, HPLC analyses are usually carried out at ambient temperature; scaling up to semi-preparative analysis is straightforward; volatile and non-volatile compounds can be analysed in a single HPLC run; for simple separation problems, rapid analyses are possible.

Depending on the nature of compounds to be analysed, several scenarios for applications of HPLC in aroma research are imaginable: quantification of major compounds in aroma extracts or of compounds which can be detected with high sensitivity, where, in general, the method of choice is reversed-phase HPLC owing to the convenient use of aqueous mobile phases; prefractionation of aroma extracts in order to obtain less complex fractions that can be further analysed by GC or HPLC, which can be an advantageous approach in trace analysis; purification of compounds for structural investigations or use in sensory evaluation, where the starting solution will generally be an already prefractionated aroma extract.

The concentration of HPLC fractions prior to the next step of analysis necessitates their extraction when employing aqueous or other high-boiling solvents as a mobile phase. The use of highly volatile HPLC solvents greatly facilitates

* Corresponding author.

0021-9673/93/$06.00

the concentration of fractions without an important loss of aroma compounds, and also on-line coupling to capillary GC [1]. It implies, however, the normal-phase mode.

The use of polar bonded silica-based supports continues to grow. Compared with bare silica, they offer the advantage of easier handling, as the equilibration time after changing the mobile phase is much shorter (permitting gradient runs) and the mobile phase components do not have to be water saturated. In the aroma field, however, the applications of this type of column (mainly cyano) are rather scarce [2–7].

In this work, we focused on the diol support in the normal-phase mode, in view of the encouraging results obtained in our laboratory in prefractionating aroma extracts and in isolating some compounds of interest [8]. Some fundamental work aimed at understanding the mechanisms of retention on this support has already been done [9–12]. Capacity factors for selected compounds (essentially hydrocarbons and some phenols) have been reported.

The aim of this work was to determine the capacity factors of compounds within all important chemical classes present in aroma extracts of plants and foodstuffs, in order to obtain a database for the prediction of retention behaviour. Particular attention was paid to a highly volatile mobile phase system, with a view to facilitating the concentration of fractions or the recovery of pure compounds.

EXPERIMENTAL

Chemicals

Pentane was of spectroscopic grade (SDS, Peypin, France) or HPLC grade (Fisons, Loughborough, UK). Diethyl ether (HPLC grade) was obtained from SDS. Prior to use, all solvents were filtered through a 0.45-μm membrane filter (Millipore, Bedford, MA, USA).

The standards were from our laboratory collection of volatile compounds, except vitispirane, which was a gift from Dr. Y. Le Fur (ENITA, Dijon, France).

Equipment

A Varian Model 9010 gradient pump and a Varian 9050 programmable UV detector were used. Sample injection was achieved with a Gilson 232 autosampler, equipped with a Rheodyne Model 7010 injection valve. The pump head was maintained at constant temperature by a laboratory-made cooling jacket. The column was LiChrospher 100 Diol (5 μm), 250 × 4.0 mm I.D., from Merck (Darmstadt, Germany). Its temperature was controlled by a water-jacket. Both cooling devices were connected to a Maton TH 50 LF S2 cryocooler, adjusted to 15.0 ± 0.1°C. The room was air conditioned at 20°C.

The solvent bottles contained 25 g of 4 Å molecular sieve for 2.5 l of solvent and were continuously sparged with a gentle stream of helium to reduce detector noise.

The pump flow-rate was set to 1.5 ml/min and controlled by a PhaseSep (Deeside, UK) digital flow meter with analogous output. Chromatographic and flow data were recorded and treated by Coconut, a PC-based four-channel acquisition and treatment software developed in our laboratory.

Procedures

The mobile phase was pentane with varyious amounts of diethyl ether to adjust the polarity. Five different mobile phases were employed (Table I). The amounts of diethyl ether were chosen in such a manner that a capacity factor of about 10 for one compound with a defined mobile phase composition corresponds to a capacity factor of about 2 for the same compound with a mobile phase of immediately higher polarity.

A solution of each compound, at a concentration of about 200 mg/l, was prepared in pentane. Three injections were made, choosing an eluent for which the resulting capacity factor was between *ca.* 2 and 10.

Dead time determination

The dead time was obtained by measuring the retention time of the signal observed when injecting pentane on to the column equilibrated with a diethyl ether-containing mobile phase.

There are two requirements for a suitable dead time marker [13,14]: no retention under the

TABLE I

MOBILE PHASES AND STANDARD COMPOUNDS

Mobile phase no.	Pentane (%)	Diethyl ether (%)	Standard compound	$\overline{k'_S}$ [a]
1	100	0	Methyl benzoate	3.22
2	99.2	0.8	Benzaldehyde	2.18
3	95	5	Anisaldehyde	4.67
4	80	20	Indole	2.20
5	50	50	Coniferaldehyde	3.32

[a] $\overline{k'_S}$ is the mean capacity factor of the standard compound.

operating conditions, and the same extent of exclusion by the pores of the support as the solutes under investigation. Pentane approximately fulfils the first condition, but not necessarily the second, as exclusion depends on the molecule size. With regard to the range of molecule size of the solutes studied (from formaldehyde to molecules such as ethyl tetradecanoate) and to the necessity of using one dead time marker only to obtain comparable results, pentane seems a reasonable compromise. The error incurred is assumed to be negligible.

When measuring the dead time in this way for different mobile phase compositions, a decrease was observed with an increase in the amount of diethyl ether. An extrapolation allowed the determination of the corresponding dead time for pure pentane. A direct measurement is not possible because no signal is obtained on injecting pentane with pure pentane as the mobile phase. The results of the regression analysis are presented in Table II.

Calculations

Preliminary determinations of capacity factors showed that the repeatability was not always satisfactory. Over a period of some days, significant variations of the k' were observed. As virtually all chromatographic parameters that could influence the retention time have been controlled, we suspected that the percentage of diethyl ether added to pentane by the pump varied to some extent.

To correct for these variations, the concept of selectivity coefficients [15] was employed. For each mobile phase composition, a standard was chosen and co-injected with the compounds of interest. Once all injections had been accomplished, a mean capacity factor ($\overline{k'_S}$) for each standard was calculated (Table I). Taking the

TABLE II

COLUMN DEAD TIME AS A FUNCTION OF MOBILE PHASE COMPOSITION

Mobile phase no.	Diethyl ether (%)	Mean t_0 (s)	n	R.S.D. (%)	t_0 from regression analysis (s)[a]
1	0	–	–	–	114.4
2	0.8	114.3	12	1.02	114.3
3	5	113.6	15	1.81	113.8
4	20	112.0	25	0.97	111.8
5	50	107.7	9	1.67	107.8

[a] Regression data: Slope = −0.1326 (S.D. 0.0054); intercept = 114.4 (S.D. 0.208); standard error = 0.208; correlation coefficient = 0.998; number of observations = 4.

TABLE III

REPEATABILITY OF THE SELECTIVITY COEFFICIENTS

k'_C, k'_S and α are the capacity factors of the sample and the standard and their ratio, respectively. Measurements over a period of 3 days. Mobile phase, pentane–diethyl ether (99.5:0.5); sample, methyl propionate; standard, methyl cinnamate. For other operating conditions, see text.

Injection no.	k'_C	k'_S	α
1	1.45	3.71	0.392
2	1.47	3.74	0.394
3	1.48	3.75	0.396
4	1.54	3.86	0.398
5	1.54	3.86	0.398
6	1.53	3.86	0.398
7	1.57	4.06	0.387
8	1.56	4.06	0.385
9	1.55	4.04	0.384
Mean			0.393[a]

[a] R.S.D. = 1.41%.

mean capacity factor of the corresponding standard, the capacity factors for the compounds of interest were then calculated according to the equation

$$k' = \overline{k'_S} \cdot \frac{t_C f_C - t_0 f_N}{t_S f_S - t_0 f_N}$$

where t_C and t_S are the retention times of the compound and of the standard, and f_C and f_S are the mean flow-rates during elution of the compound and of the standard, respectively. The flow-rate was introduced in the calculation in order to eliminate residual flow variations we observed in spite of thermostating of the pump head. Good day-to-day repeatability was obtained, as shown in Table III.

This procedure for the determination of capacity factors is assumed to give values of higher precision, as minor fluctuations of the mobile phase composition and of the flow-rate are largely corrected for.

RESULTS AND DISCUSSION

The compounds used in this work were chosen within the chemical classes commonly contained in the volatiles fraction of plants and foodstuffs, *i.e.*, hydrocarbons, alcohols, phenols, aldehydes, ketones, esters, sulphur- and nitrogen-containing compounds and heterocyclics.

Two classes of compounds, acids and amines, exhibited a particular elution behaviour. Even though elution of carboxylic acids with a moderately polar mobile phase [pentane–diethyl ether (80:20)] was possible, successive injections decreased their retention and the peak shape degraded markedly. Moreover, the retention times of neutral compounds were affected after injection of acids. This effect was reversible; the initial state of the column could be re-established by flushing it with diethyl ether. Aliphatic amines gave rise to the same phenomena, confirming observations of Smith and Cooper [10].

We attributed this behaviour to partial adsorption of the compounds on active sites of the support. Addition of a polar modifier in small amounts to the mobile phase would certainly eliminate this problem, but would also be detrimental to its volatility. Taking into account the objectives of this work, strongly acidic and basic compounds were not considered further.

Discussion of k' values

The k' values obtained as described above are given in Table IV and Fig. 1 shows their subdivision according to the chemical classes.

Even the most polar compounds under investigation, *e.g.*, veratryl alcohol, maltol and sinapaldehyde, which are likely to be irreversibly adsorbed on silica, can be eluted with pentane–diethyl ether (50:50). On the other hand, very non-polar compounds (hydrocarbons, thiols, thioethers, furans and thiophenes) undergo no or only slight retention, precluding the separation of individual compounds. Most classes of volatiles, however, are situated between these two extremes.

The presence or absence of a hydroxyl group (or, to a lesser extent, an aromatic methoxy group) essentially governs retention. In the absence of such a group, the retention increases in the order hydrocarbons < ethers < aldehydes < esters < ketones < nitriles for comparable molecular structures (same chain length). The corresponding retention order on bare silica [16], hydrocarbons < ethers < nitriles < esters <

TABLE IV

CAPACITY FACTORS OF AROMA COMPOUNDS ON DIOL

For compositions of mobile phases, see Table I.

Compound	Mobile phase	k'
Hydrocarbons		
Camphene	1	<0.5
Myrcene	1	<0.5
Benzene	1	<0.5
Styrene	1	<0.5
Naphthalene	1	<0.5
Phenanthrene	1	1.19
Saturated alcohols		
Ethanol	4	3.40
1-Propanol	4	3.14
1-Butanol	4	2.96
1-Pentanol	4	2.82
1-Hexanol	4	2.74
1-Octanol	4	2.61
1-Dodecanol	4	2.41
2-Butanol	4	2.58
2-Pentanol	4	2.39
3-Octanol	3	8.34
2-Methyl-1-butanol	4	2.20
3-Methyl-1-butanol	4	2.75
2-Methyl-2-butanol	4	2.39
3-Methyl-2-butanol	4	1.99
Menthol	4	1.85
Thujyl alcohol	3	2.07
Fenchyl alcohol	3	4.19
Isolongifolol	4	3.07
Myrtanol	4	3.33
Unsaturated alcohols		
2-Buten-1-ol	4	3.16
(*E*)-2-Hexen-1-ol	4	2.89
(*Z*)-3-Hexen-1-ol	4	2.92
(*E*)-2-Octen-1-ol	4	2.72
(*Z*)-3-Octen-1-ol	4	2.68
(*Z*)-6-Nonen-1-ol	4	3.22
(*E*,*Z*)-2,6-Nonadien-1-ol	4	2.99
1-Penten-2-ol	3	7.20
1-Hexen-2-ol	3	6.80
1-Octen-2-ol	2	9.52
α-Terpineol	4	2.38
4-Terpineol	3	4.36
p-Menth-1-en-9-ol	4	2.71
Isopulegol	3	5.03
Dihydrocarveol	4	2.37
Pinocarveol	3	6.00
Myrtenol	4	2.37
Carveol	4	2.25
1,8-Menthadien-4-ol	3	5.69
α-Bisabolol	3	6.84
Guaiol	3	10.21
8(15)-Cedren-9-ol	4	2.13
Geraniol	4	3.69
Nerol	4	3.17
Citronellol	4	3.00
Linalool	3	5.38
Lavandulol	3	5.18
Farnesol	4	3.05
(*Z*)-Nerolidol	3	4.97
(*E*)-Nerolidol	3	5.10
Aromatic alcohols		
Benzyl alcohol	4	3.88
2-Phenylethanol	4	4.52
Cinnamyl alcohol	4	5.42
Anisyl alcohol	4	3.95
Veratryl alcohol	5	5.30
p-Cymen-7-ol	4	3.61
p-Cymen-8-ol	4	2.40
Furfuryl alcohol	4	4.15
Phenols		
Phenol	4	2.01
2-Methylphenol	3	7.21
3-Methylphenol	4	1.99
4-Methylphenol	4	1.99
4-Ethylphenol	4	1.92
5-Isopropyl-2-methylphenol	3	5.67
4-Vinylphenol	4	1.58
2-Methoxyphenol	3	4.40
2-Methoxy-4-vinylphenol	4	1.57
2-Methoxy-4-allylphenol	3	4.43
2-Methoxy-4-(1-propenyl)phenol	4	1.86
2,6-Dimethoxyphenol	5	3.33
Aliphatic ethers		
Diethyl ether	1	1.09
Vitispirane	1	1.15
Aromatic ethers		
Methoxybenzene	1	0.74
1,4-Dimethoxybenzene	1	3.83
Epoxides		
β-Caryophyllene oxide	2	3.53
Limonene-1,2-epoxide	1	4.84
Thiols		
Butanethiol	1	<0.5

(Continued on p. 312)

TABLE IV *(continued)*

Compound	Mobile phase	k'
Aliphatic saturated aldehydes		
Formaldehyde	4	1.59
Propionaldehyde	2	1.92
Butyraldehyde	1	2.22
Nonanal	1	1.65
Aliphatic unsaturated aldehydes		
Propenal	2	2.89
2-Butenal	2	5.39
2-Pentenal	2	3.58
(*E*)-2-Hexenal	2	3.02
(*E*)-2-Nonenal	2	2.70
(*Z*)-6-Nonenal	1	4.56
(*E*,*E*)-2,6-Nonadienal	2	3.18
Geranial	2	5.49
Neral	2	5.16
Citronellal	1	2.61
3-Cyclocitral	1	1.80
Perillaldehyde	2	3.13
Myrtenal	2	2.51
Phenylacetaldehyde	3	4.99
Aromatic aldehydes		
Benzaldehyde	2	2.18
4-Methylbenzaldehyde	2	2.55
Cinnamaldehyde	2	7.46
α-Amylcinnamaldehyde	2	2.72
Aromatic hydroxylated aldehydes		
4-Hydroxybenzaldehyde	4	9.32
Anisaldehyde	3	4.67
Vanillin	4	7.03
Piperonal	3	4.66
Coniferaldehyde	5	3.32
Sinapaldehyde	5	6.72
Heterocyclic aldehydes		
Furfural	3	4.35
5-Methylfurfural	3	4.53
5-Hydroxymethylfurfural	5	4.62
2-Formylpyrrole	4	4.65
Aliphatic saturated ketones		
Acetone	3	4.68
2-Pentanone	2	2.82
2-Hexanone	2	2.65
2-Heptanone	2	2.52
2-Nonanone	2	2.42
2-Undecanone	2	2.37
5-Undecanone	1	3.17
Camphor	2	3.43
Menthone	1	2.42
3-Thujone	2	1.95
Aliphatic unsaturated ketones		
5-Hexen-2-one	2	2.94
6-Methyl-5-hepten-2-one	2	2.84
6-Methyl-3,5-heptadien-2-one	3	3.84
2,6-Dimethyl-2,5-heptadien-4-one	2	1.96
Artemisia ketone	1	2.20
Carvone	2	4.43
Dihydrocarvone	2	2.61
α-ionone	2	4.41
β-ionone	2	5.40
cis-Jasmone	3	6.53
Nootkatone	3	7.28
Piperitone	3	3.25
Pulegone	2	4.13
Verbenone	3	6.77
Damascenone	2	2.99
Geranylacetone	2	3.03
Isophorone	2	4.84
Aromatic ketones		
Acetophenone	2	3.73
Indonone	3	5.16
Benzyl methyl ketone	2	6.31
Heterocyclic ketones		
2-Acetylfuran	3	4.27
2-Acetylpyridine	3	4.20
2-Acetylpyrrole	4	4.80
2-Acetylthiazole	3	3.11
Aromatic hydroxylated ketones		
3-Hydroxy-4-phenyl-2-butanone	2	9.49
Anisylacetone	3	5.28
Propiovanillone	4	5.11
Acetosyringone	5	4.61
Acetovanillone	4	7.74
Aliphatic hydroxylated ketones		
Acetoin	4	5.00
3-Hydroxy-3-methyl-2-butanone	4	2.40
4-Hydroxy-4-methyl-2-pentanone	4	5.44
4-Hydroxy-2,5-dimethyl-3-hexanone	3	2.19
Epoxy-β-ionone	3	3.21
Aliphatic diketones		
Diacetyl	2	1.69
Pentane-2,4-Dione	2	2.79
Hexane-2,5-Dione	4	4.37
Miscellaneous cyclic enones		
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	2	3.60
3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone	5	3.34
2-Hydroxy-3-methyl-2-cyclopenten-1-one	4	4.40

TABLE IV *(continued)*

Compound	Mobile phase	k'
Maltol	5	6.07
Diosphenol	2	2.89
Mesifuran	3	3.22
Saturated esters		
Methyl acetate	1	5.40
Methyl propionate	1	3.58
Methyl butyrate	1	2.71
Methyl hexanoate	1	2.50
Methyl octanoate	1	2.40
Methyl tetradecanoate	1	2.31
Ethyl formate	1	2.47
Ethyl acetate	1	5.42
Ethyl propionate	1	3.00
Ethyl butyrate	1	2.68
Ethyl hexanoate	1	2.51
Ethyl heptanoate	1	2.30
Ethyl octanoate	1	2.42
Ethyl tetradecanoate	1	2.29
Propyl hexanoate	1	2.01
Butyl pentanoate	1	1.91
Pentyl butyrate	1	2.14
Hexyl propionate	1	2.29
Pentyl pentanoate	1	1.98
Isopentyl pentanoate	1	1.89
Pentyl isopentanoate	1	1.86
Pentyl 2-methylbutyrate	1	1.75
Isopentyl 2-methylbutyrate	1	1.68
Isopentyl isopentanoate	1	1.72
Bornyl acetate	1	1.87
Fenchyl acetate	1	2.02
Menthyl acetate	1	2.86
Aliphatic unsaturated esters		
Ethyl (*E*)-2-butenoate	1	4.71
Ethyl (*E*)-2-octenoate	1	3.46
Ethyl (*E*)-2-decenoate	1	3.38
Ethyl (*E*)-4-decenoate	1	2.71
Ethyl (*Z*)-4-decenoate	1	2.55
Vinyl acetate	1	1.50
(*E*)-2-Hexenyl acetate	1	3.52
1-Octen-3-yl acetate	1	2.85
Citronellyl acetate	1	3.80
Geranyl acetate	1	5.07
Linalyl acetate	1	3.38
Perillyl acetate	1	5.75
Carvyl acetate	1	3.51
Terpinyl acetate	1	3.49
Aromatic esters		
Benzyl acetate	1	6.90
2-Phenylethyl acetate	2	3.01
1-Phenylethyl acetate	1	6.56
3-Phenylpropyl acetate	2	3.21
Methyl benzoate	1	3.22
Ethyl benzoate	1	2.96
Methyl cinnamate	2	3.38
Aromatic hydroxylated esters		
Methyl salicylate	1	1.66
Methyl vanillate	4	4.99
Methyl anthranilate	3	4.37
Heterocyclic esters		
Methyl nicotinate	4	4.06
Aliphatic hydroxylated esters		
Ethyl lactate	4	2.74
Ethyl 3-hydroxybutyrate	4	4.48
Ethyl 2-hydroxyhexanoate	3	6.14
Diethyl tartrate	5	3.16
Keto esters		
Ethyl acetoacetate	3	2.96
Diesters		
Diethyl malonate	3	2.87
Diethyl succinate	3	2.90
Triglycerides		
Tributyrin	3	5.11
Tristearin	3	9.10
Saturated lactones		
γ-Butyrolactone	2	6.47
γ-Decalactone	2	6.42
δ-Decalactone	2	6.38
β-Methyl-γ-octalactone	2	6.43
Unsaturated lactones		
α-Methylene-γ-butyrolactone	2	6.46
β-Angelica lactone	1	4.48
6-Pentyl-2-pyrone	3	9.65
Nitriles		
Acetonitrile	4	3.45
Allyl cyanide	2	2.86
Benzyl cyanide	3	3.57
Cyanohydrins		
α-Hydroxyphenylacetonitrile	4	5.94
Isothiocyanates		
Butyl isothiocyanate	1	0.54
2-Propenyl isothiocyanate	1	0.81
2-Phenylethyl isothiocyanate	1	1.73
Furans		
Menthofuran	1	<0.5
Pyridines		
Pyridine	5	3.50
Quinoline	5	2.26
Pyrroles		
Pyrrole	3	3.90
Indole	4	2.20

(Continued on p. 314)

TABLE IV *(continued)*

Compound	Mobile phase	k'	Compound	Mobile phase	k'
Pyrazines			*Thiazoles*		
Pyrazine	5	2.28	Thiazole	4	2.38
Methylpyrazine	4	3.91	2,4-Dimethylthiazole	3	5.19
Ethylpyrazine	4	2.47	2-Isobutylthiazole	3	3.20
2,3-Dimethylpyrazine	4	3.94	4-Methyl-5-vinylthiazole	4	1.77
2,5-Dimethylpyrazine	4	3.42	Benzothiazole	4	1.84
Tetramethylpyrazine	4	2.78	*Thiophenes*		
Methoxypyrazine	3	3.29	Thiophene	1	<0.5
2-Isobutyl-3-methoxypyrazine	3	1.79	2,5-Dimethylthiophene	1	<0.5

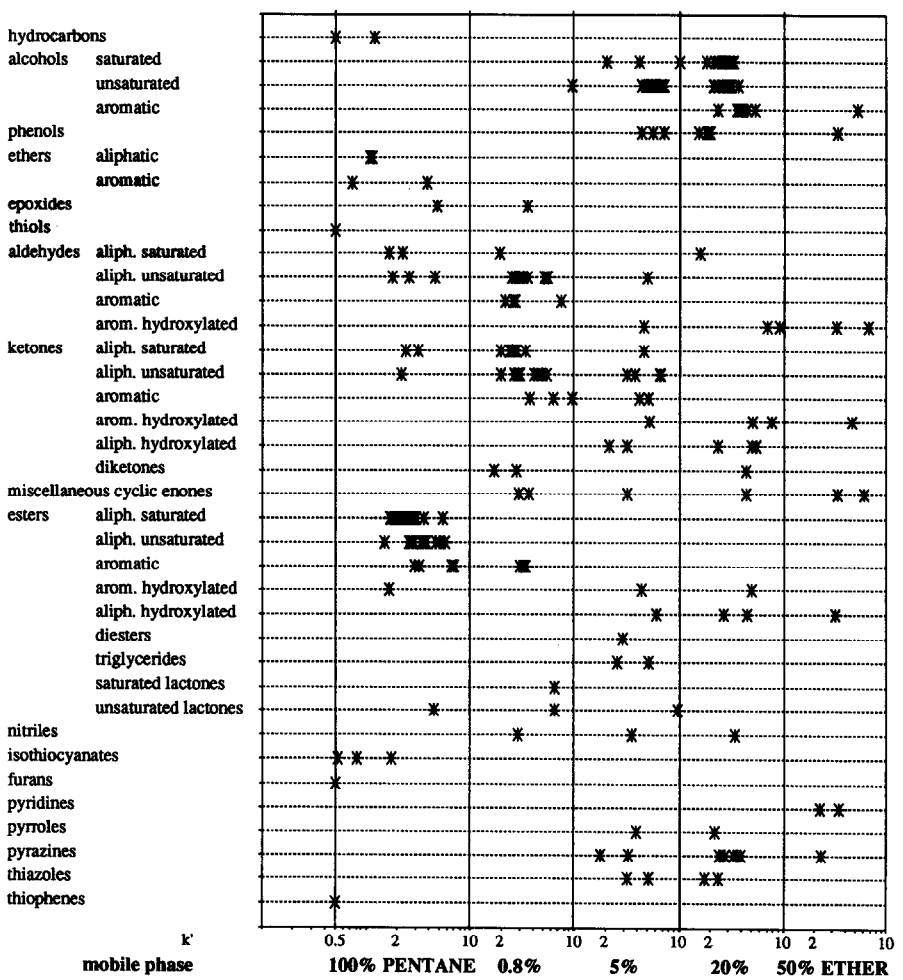

Fig. 1. Subdivision of the capacity factors of the compounds under investigation according to chemical classes. One asterisk corresponds to the capacity factor of one compound. The abscissa shows the mobile phase compositions with increasing polarity. Within each mobile phase, the asterisks are positioned on a scale of k' from 1 to 10 (from 0.5 to 10 for pure pentane). The corresponding values are given in Table IV.

ketones < aldehydes, shows that there are substantial differences in selectivity for aldehydes and nitriles on these two stationary phases.

Within homologous series, as expected, an increase in retention is observed with decreasing alkyl chain length. The "shortest" aldehyde and ketone, formaldehyde and acetone, are strongly retained. The smallest aliphatic esters, apart from the gaseous methyl formate, are methyl acetate and ethyl formate. These two differ markedly in retention, ethyl formate ($k' = 2.47$) being less retained than methyl acetate ($k' = 5.40$). Comparison with ethyl acetate ($k' = 5.42$) (all three values for pure pentane as mobile phase) reveals that the formate does not follow the postulated rule.

On examination of a series of aliphatic esters with equal chain length of nine carbons, differing only in the position of the –COO– group, a minimum of retention can be found when the carbonyl group is located in the centre of the molecule (Fig. 2). Maximum steric hindrance of the functional group is considered to be responsible for this behaviour.

Branching of an alkyl chain decreases retention (see, *e.g.*, pentyl pentanoate and some of its methyl branched isomers, and 1-pentanol, 2-pentanol and their branched isomers). In addition, retention decreases when the methyl side-chain approaches the functional group. However, the tertiary alcohol 2-methyl-2-butanol is more retained than its secondary homologue, 3-methyl-2-butanol.

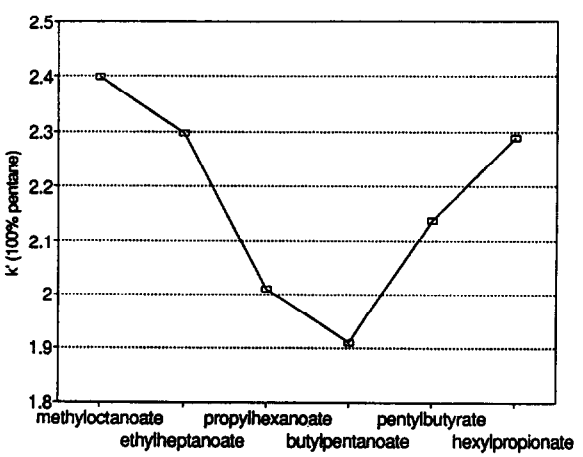

Fig. 2. Capacity factors of a series of aliphatic esters of nine carbons, differing only in the position of the functional group. The mobile phase is pure pentane.

The retention of the unsubstituted heterocyclics under investigation follows the order furan ≈ thiophene ≪ pyrrole < thiazole < pyrazine < pyridine. Only the nitrogen-containing rings are markedly retained.

In order to evaluate the capability of the diol column for the preseparation of aroma compounds from triglycerides, the retention of tributyrin and tristearin was studied. These two compounds, encompassing approximately the range of triglycerides present in nature, are eluted between aldehydes, ketones, lactones and nitriles on one hand, and alcohols, phenols, nitrogen-containing heterocyclics and hydroxylated compounds on the other, interfering with poorly retained unsaturated alcohols and phenols. With the exception of the latter compounds, aroma extract preseparation on diol seems to be possible in the presence of larger amounts of triglycerides.

CONCLUSIONS

The diol support is less retentive than silica gel, which means that complete elution under normal-phase conditions is possible even for the most polar compounds usually present in aroma extracts. With the exception of strong acids and strong bases, no irreversible adsorption was observed. Higher selectivity, and hence a higher separation power, is exhibited for compounds containing a hydroxyl group or a nitrogen, followed by those containing a carbonyl group. The lack of retention for non-polar compounds such as hydrocarbons can be advantageous for aroma extract preseparations. After recovery of this fraction, eluted nearly at the dead time, separation could be achieved on an appropriate support.

Other supports are currently under investigation. The results will be compared with those obtained on the diol support and published later.

ACKNOWLEDGEMENT

We are grateful to Robert Almanza for developing the software allowing the automation of the calculations in this work.

REFERENCES

1 C.G. Chappell, C.S. Craeser and M.J. Shepherd, presented at the *19th International Symposium on Chromatography, Aix-en-Provence, September 1992.*
2 C.L. Teitelbaum, *J. Agric. Food Chem.*, 25 (1977) 466–470.
3 J.A. Schmit, R.C. Williams and R.A. Henry, *J. Agric. Food Chem.*, 21 (1973) 551–556.
4 L.W. Wulf, C.W. Nagel and A.L. Branen, *J. Chromatogr.*, 161 (1978) 271–278.
5 H.H. Wisnieski, R.L. Yates and H.M. Davis, *J. Chromatogr.*, 255 (1983) 455–461.
6 M. Benincasa, F. Buiarelli, G.P. Cartoni and F. Coccioli, *Chromatographia*, 30 (1990) 271–276.
7 F. Buiarelli, G.P. Cartoni, F. Coccioli and E. Ravazzi, *Chromatographia*, 31 (1991) 489–492.
8 D. Barron, *Z. Lebensm.-Unters.-Forsch.*, 193 (1991) 454–459.
9 A.M. Siouffi, M. Righiezza and G. Guiochon, *J. Chromatogr.*, 386 (1986) 189–202.
10 P.L. Smith and W.T. Cooper, *J. Chromatogr.*, 410 (1987) 249–265.
11 P.L. Smith and W.T. Cooper, *Chromatographia*, 25 (1988) 55–60.
12 A.W. Salotto, E.L. Weiser, K.P. Caffey, R.L. Carty, S.C. Racine and L.R. Snyder, *J. Chromatogr.*, 498 (1990) 55–65.
13 R.J. Smith, C.S. Nieass and M.S. Wainwright, *J. Liq. Chromatogr.*, 9 (1986) 1387–1430.
14 A. Aldehai, D.E. Martire and R.P.W. Scott, *Analyst*, 114 (1989) 869–875.
15 W.W. Christie and B. Nikolova-Damyanova, *presented at the AOCS Annual Meeting, Toronto, May 1992.*
16 H. Engelhardt and H. Elgass, in C. Horvath (Editor), *High Performance Liquid Chromatography*, Vol. 2, Academic Press, New York, 1980, p. 57.