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STUDY OF RETENTION BEHAVIOUR OF ALKYLPHENOLS IN STRAIGHT- AND REVERSED-PHASE LIQUID CHROMATOGRAPHY AND APPLICATION TO THE ANALYSIS OF COMPLEX PHENOLIC MIXTURES IN CONJUNCTION WITH GAS CHROMATOGRAPHY

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SUMMARY

A series of alkyl-substituted phenols were chromatographed on four liquid chromatographic columns: three straight-phase systems on nitrile phases and one reversed-phase system on octadecylsilane. On the nitrile phases, the alkylphenols are eluted in the order di-*ortho*-, mono-*ortho*- and non-*ortho*-substituted phenols, while on the reversed-phase system it is primarily the number of substituted alkyl carbon atoms that determine the retention. Two-column plots, *i.e.*, log k' on reversed phase *versus* log k' on straight phase, give three well separated straight lines representing the di-, mono- and non-*ortho*-substituted phenols. Combination of straight- and reversed-phase liquid chromatography and capillary column gas–liquid chromatography provides an effective means for the separation and identification of complex mixtures of alkylphenols.

INTRODUCTION

Alkylphenols have considerable industrial importance and frequently appear as pollutants in the environment. Consequently, methods for the separation and analysis of alkylphenols have been much studied and numerous papers have been published. Gas–liquid chromatography (GLC) has been the preferred separation method[1–9], but also various types of mainly classical liquid chromatography (LC) have been employed[10–16].

Although very refined separations of alkylphenols can be achieved by using GLC, especially on open-tubular columns[4,5,9], the structural information contained in chromatograms is often difficult to analyse owing to the simultaneous influence of vapour pressure and polar interactions[17].

In liquid chromatography, the relationship between retention and structure of alkylphenols is often more clear-cut. Thus, it has been shown by classical column adsorption chromatography and thin-layer chromatography (TLC) using the silica–benzene system[10–13] that alkylphenols can be distinguished according to *ortho*-substitution. Liquid–liquid chromatography (LLC) of alkylphenols has also been

performed on amide-impregnated cellulose and the retention shown to be structure related[14,15]. Other types of LC which, although less important for this purpose, have been used for the separation and identification of alkylphenols are ion-exchange[18,19] and paper chromatography[16].

Modern liquid chromatography has provided a further technique for alkylphenol separations[20–24]. However, no extensive study of the retention behaviour of alkylphenols using modern liquid chromatographic methods has been published so far.

The aim of this work was to make a detailed chromatographic investigation of a number of alkylphenols using straight- and reversed-phase LC in order to be able to analyze complex mixtures. The investigation also included gas chromatography (GC), and a procedure is presented for the separation of complex mixtures of alkylphenols by a combination of chromatographic methods.

EXPERIMENTAL

Liquid chromatographic apparatus

The LC pump used was a Varian Model 4100 (Varian, Palo Alto, Calif., U.S.A.) and the detectors were a Laboratory Data Control Model 1285 UV monitor (Laboratory Data Control, Riviera Beach, Fla., U.S.A.) and a Perkin-Elmer LC-55 spectrophotometer (Perkin-Elmer, Norwalk, Conn., U.S.A.). Sample application was accomplished either by a septum injector or a valve injector (Rheodyne, Berkeley, Calif., U.S.A.). The conventionally coated liquid phases were applied by the evaporation technique on the support material Porasil C (Waters Assoc., Milford, Mass., U.S.A.; 37–73 μm), which was then dry-packed in precision-bore stainless-steel columns (400 mm $\times$ 1/4 in. O.D. $\times$ 2.1 mm I.D.) by the "tap-and-fill" method. A pre-column (400 $\times$ 5 mm I.D.) was packed with 80–100-mesh Chromosorb W coated with 30% of stationary phase. The columns were water-jacketed and thermostated to 20.0 $\pm$ 0.1°.

The bonded-phase columns were the commercially available pre-packed microparticulate columns Cyano Sil-X-I (13 $\pm$ 5 μm; 500 $\times$ 2.1 mm; Perkin-Elmer) and μBondapak C_{18} (10 $\pm$ 2 μm; 300 $\times$ 4 mm; Waters Assoc.). These columns were used at room temperature.

Gas chromatography

The GC analysis was performed on a Varian 1400 gas chromatograph equipped with a flame-ionization detector. A 1000 $\times$ 2 mm stainless-steel column was packed with Chromosorb G HP, 80–100 mesh, coated with 5% of the stationary phase [1,2,3-tris(cyanoethoxy)propane]. The column was operated at 160° at a flow-rate of 30 ml/min. The sample size injected was about 0.3 μg. The capillary Apiezon L column (Perkin-Elmer) was 150 ft. long and operated at 140° at a flow-rate of 2 ml/min. The total injected sample size was about 0.1 μg and the splitting ratio was 1:100.

Thin-layer chromatography

The TLC plates used were Camag D-B pre-coated silica gel (Camag, Muttenz, Switzerland). The thin-layer chromatograms were evaluated by means of a Perkin-

Elmer MPF-2A fluorescence spectrophotometer equipped with a 018-0057 attachment for TLC.

Chemicals

Isooctane (2,2,4-trimethylpentane; Fisher Scientific, Fairlawn, N.J., U.S.A.), methanol (analytical-reagent grade, May & Baker, Dagenham, Great Britain), ethanol (absolute, 99.5%,: Kemetylprodukter, Bromma, Sweden) and 2-propanol (pro analysi, E. Merck, Darmstadt, G.F.R.) were used. The alkylphenols were obtained from different manufacturers and were generally of 99% purity or better.

The liquid stationary phases used were 1,2,3-tris(cyanoethoxy)propane(TCEP; Varian Assoc.) and Fractonitril VI (1,2,3,4,5,6-hexakis(2'-cyanoethoxy)hexane) (Merck).

N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA; specially purified grade, Pierce, Rockford, Ill., U.S.A.) was used for the silylation experiments.

All chemicals were used without further purification.

Procedure

Alkylphenols were dissolved in the mobile phase to give concentrations of 0.1–0.5 mg/ml and 5 μl of the solutions were injected on to the LC columns. The capacity factor was determined from a mean value of three injections with a relative standard deviation of about 2%. Silylation of the phenols for the GC investigation was performed by adding a few drops of BSTFA to one drop or a small crystal of the phenol in a centrifuge tube and heating for a few minutes. Sterically hindered phenols required a longer reaction time (15–20 min). Complete silylation was achieved for all phenols except for the 2,6-di-*tert*.-butylphenols, which underwent a 50% conversion.

In the separation of alkylphenols by a combination of straight- and reversed-phase LC, a mixture of phenols was chromatographed on a Cyano Sil-X-I column. Collected fractions were evaporated nearly to dryness by a gentle stream of nitrogen and injected on to the reversed-phase column. In the combination of LC and GC for a separation, the phenols were chromatographed on the Cyano Sil-X-I column. The fractions collected were evaporated as above and a few drops of the silylation reagent were added. After a few minutes of heating, the sample was injected on to the Apiezon L capillary column.

RESULTS AND DISCUSSION

Liquid chromatography

Kirkland[20] demonstrated the analytical possibilities for alkylphenols in modern liquid chromatography by separating a mixture of phenol and six methylphenols on an "ether"-bonded phase, using 2.5% (v/v) methanol in cyclopentane as the mobile phase Sleight[21] studied solute–column interactions for phenols with various substituted groups, including methyl groups, on Durapak OPN, Carbowax 400 and Corasil II. He concluded that the retention was predominantly affected by hydrogen bonding and that for *ortho*-alkyl-substituted phenols the retention seemed to be related to the size of the alkyl group. Nitrile phases were used by Huber[22] and Karger *et al.*[23] for the separation of some alkyl-substituted phenols.

In Table I the experimental conditions for the LC systems are summarized. The column chromatographic investigation included three straight-phase LC systems on nitrile phases, two of which were conventionally coated and one chemically bonded, and one reversed-phase system on a chemically bonded octadecylsilane phase. The TLC separation on the silica gel–benzene system was included in order to compare the retention behaviour of some alkylphenols on the nitrile phases with that on silica gel.

TABLE I

OPERATING CONDITIONS FOR THE LIQUID CHROMATOGRAPHIC SYSTEMS

Support (particle size)	*Stationary phase (surface coverage)*	*Mode*	*Column dimensions (mm)*	*Eluent*	*Flow velocity ($mm \cdot sec^{-1}$)*	*Pressure (atm)*	*HETP (mm)*	*Asymmetry factor*
Porasil (37–74 μm)	TCEP*	LLC	400 × 2.1	Isooctane	2.4	20	1	0.9
Porasil (37–74 μm)	Fractonitril VI*	LLC	400 × 2.1	Isooctane	2.4	20	1	0.8
Sil-X-I (13 ± 5 μm)	Cyano Sil-X-I**	BPC***	500 × 2.6	Isooctane + 0.5% 2-propanol	2.5	30	0.5	0.8
μPorasil (10 ± 2 μm)	μBondapak C_{18} (ca. 10%)	BPC***	300 × 4.0	Ethanol–water (60:40)	0.6	50	0.1	0.9

* Equilibrium Loading.
** Surface coverage unknown.
*** Bonded-phase chromatography.

The results from the different LC systems are given in Table II in terms of the capacity factor, k'. In the TLC investigation the R_F values were measured and the capacity factors calculated from the relationship

$$k'_{TLC} = \left(\frac{1}{R_F} - 1\right) \tag{1}$$

It should be noted that for low R_F values a change in R_F within the experimental error (± 0.02) will result in a large change in k'_{TLC}.

Straight-phase LLC and TLC. The alkylphenols in Table II are arranged into three structural classes according to the substitution pattern at the *ortho* positions, *viz.*, di-*ortho*-, mono-*ortho*- and non-*ortho*-substituted phenols. Within each structural group the phenols are arranged in order of decreasing k'-values on the TCEP stationary phase. It can be seen that the correlation between retention and structural class holds for all but one of the phenols investigated (3,5-di-*tert.*-butylphenol) in chromatography on the conventionally coated nitrile phases, and all but two (3,5- and 2,5-di-*tert.*-butylphenol) on the chemically bonded phase. Comparison with the k'_{TLC} values shows that in this instance there is a considerable overlap between retention values of mono- and di-*ortho*-substituted compounds, in that phenols with one large *ortho*-situated alkyl group, *e.g.*, *tert.*-butyl, occurs among the di-*ortho*-substituted compounds.

Division into structural classes, on the TLC separation of alkylphenols, is

considered to be caused mainly by differences in the strength of hydrogen bonding between the phenolic hydroxyl group and the adsorbent, owing to steric hindrance from *ortho*-situated alkyl groups. This is indicated *inter alia* by analogy with the magnitude of the infrared shift of the hydroxyl absorption band, due to intermolecular hydrogen bonding[25]. In the same way, differences in strength of hydrogen bonding to the proton-accepting cyano groups in the nitrile phase are considered to be mainly

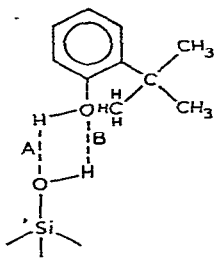
TABLE II

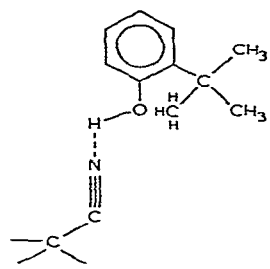
CAPACITY FACTORS, k', FOR ALKYLPHENOLS IN FOUR COLUMN LIQUID CHROMATOGRAPHIC SYSTEMS (FOR CONDITIONS, SEE TABLE I) AND ONE TLC SYSTEM (SILICA GEL–BENZENE)

No.	*Substituent*	*Capacity factor, k'*				k'_{TLC}: SiO_2 (LSC)
		TCEP (LLC)	*Fracto-nitril (LLC)*	*Cyano Sil-X-I (BPC)*	*μBondapak C_{18} (BPC)*	
	Di-ortho-substituted					
1	2,6-Di-*tert.*-butyl-4-methyl	u*	u	u	9.41	0.0
2	2,6-Di-*tert.*-butyl	u	u	u	7.40	0.0
3	2,6-Di-*sec.*-butyl	0.40	0.59	0.43	5.11	—
4	2,6-Diisopropyl	0.67	0.83	0.78	2.58	—
5	2,3,5,6-Tetramethyl	1.4	1.6	1.6	1.26	—
6	2,4,6-Trimethyl	1.5	1.9	2.2	0.96	1.6
7	2,6-Dimethyl	1.9	2.0	2.6	0.69	1.3
	Mono-ortho-substituted					
8	2,5-Di-*tert.*-butyl	2.3	2.8	1.8	6.04	—
9	2-*tert.*-Butyl-5-methyl	3.4	3.9	—	2.50	0.7
10	2-*tert.*-Butyl-4-methyl	3.8	4.4	3.2	2.28	0.7
11	2-*tert.*-Butyl	4.9	5.7	4.0	1.87	—
12	2,4,5-Trimethyl	4.9	5.7	5.3	1.00	3.0
13	2,3,5-Trimethyl	5.2	5.9	5.0	0.95	2.5
14	2-Propyl	5.5	6.1	5.5	1.14	—
15	2,5-Dimethyl	5.6	6.0	6.3	0.69	3.0
16	2-Isopropyl	5.7	6.3	5.5	1.07	—
17	2,4-Dimethyl	6.2	6.6	6.5	0.69	2.9
18	2-Ethyl	6.4	7.0	6.0	0.74	2.3
19	2,3-Dimethyl	7.0	7.5	6.5	0.69	2.5
20	2-Methyl	7.6	8.2	7.2	0.44	3.0
	Non-ortho-substituted					
21	3,5-Di-*tert.*-butyl	6.4	7.5	6.0	3.73	—
22	4-*tert.*-Pentyl	9.7	12.0	8.8	1.65	4.9
23	4-*sec.*-Butyl	9.9	13.0	9.1	1.36	4.3
24	3,5-Dimethyl	10.1	11.1	10.1	0.64	5.7
25	3,4,5-Trimethyl	—	12.0	—	0.79	—
26	3-Ethyl	10.7	12.2	9.8	0.67	4.9
27	4-*tert.*-Butyl	10.7	12.7	9.4	1.21	—
28	4-Propyl	10.8	12.3	10.0	1.01	—
29	3,4-Dimethyl	11.7	12.4	10.3	0.57	4.9
30	4-Ethyl	11.7	13.9	10.9	0.66	4.9
31	3-Methyl	12.8	13.7	11.3	0.44	5.3
32	4-Methyl	13.0	14.1	11.4	0.42	5.7
33	Phenol	17.0	17.0	12.7	0.28	5.3

* u = Unretained.

responsible for the division into structural groups on the nitrile phase. The hydrogen-bond interaction between phenols and silica gel and a nitrile phase, respectively, can be illustrated as follows:





The surface silanol groups are capable of forming hydrogen bonds with both the oxygen and the hydrogen atom of the phenolic hydroxyl group[26], although the former interaction (marked B) is the one generally considered[27,28]. The cyano group, on the other hand, is only capable of forming a hydrogen bond to the hydrogen atom of the phenolic hydroxyl group. As indicated, a bulky *ortho*-substituent, *e.g.*, *tert.*-butyl, should interfere to a greater extent with the dominating B-type hydrogen bonding to the silanol group than with the hydrogen bonding to the cyano group, thus explaining the observed differences in selectivity.

For the nitrile phases, adsorption to silanol groups on the relatively active silica gel support material, Porasil C[29], also contributes to retention, as indicated by Fig. 1, where capacity factors for 2,6-, 2,3- and 2,5-dimethylphenols are plotted against the loading of TCEP. The curves show the typical picture of a mixed-mechanism chromatographic system[30]. Below 10% loading, the retention rises due to increased adsorption to the support, and above 10% the retention increase is mainly caused by the increase in phase volume ratio:

$$k' = (V_S \cdot V_M^{-1}) \cdot D_A^{-1} \qquad (2)$$

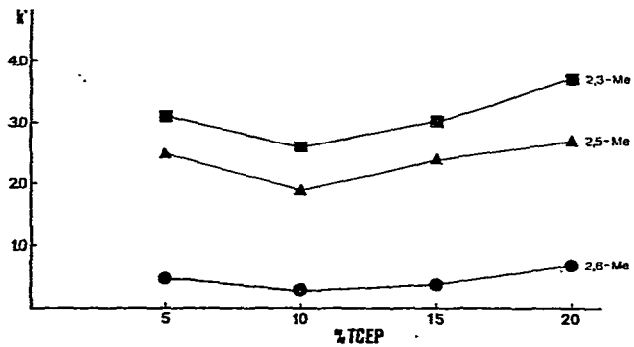


Fig. 1. Capacity factors, k', for 2,3-, 2,5- and 2,6-dimethylphenol as a function of liquid loading (% TCEP). Support, Porasil C; mobile phase, isooctane.

where D_A = distribution coefficient and $V_S \cdot V_M^{-1}$ = phase volume ratio. The variation in k' with loading is less pronounced for 2,6-dimethylphenol where hydrogen bonding is weakened by steric hindrance. The capacity factor in an idealized mixed mechanism can be expressed as[31]

$$k' = (A_S \cdot V_M^{-1}) K_{ads.} + (V_S \cdot V_M^{-1}) D_A^{-1} \quad (3)$$

where A_S = surface area of the support and $K_{ads.}$ = the adsorption coefficient. The retention data in Table II refer to the steady state liquid loading for the conventionally coated phases[30] after equilibration for at least 24 h. Certainly, the separation efficiency of the LLC-systems can be much improved by the use of a microparticulate support.

The variation of k' for the nitrile phases within each structure group is demonstrated in Fig. 2, where log k' is plotted against alkyl carbon number for the four different LC systems. It can be seen that for the straight-phase systems, there is a tendency for the points to collect around three stragiht lines, which coincide with the structure classes of di-*ortho*-, mono-*ortho*- and non-*ortho*-substitution.

Several types of interactions are likely to be involved in the partition of solutes between the stationary nitrile phases and the mobile phase. The significance of hydrogen bonding to the nitrile phase, for the division into structural classes, has already been discussed. Naturally, variation in the strength of these forces are also important for the change in k' within each class, especially for the mono- and di-*ortho*-substituted phenols. For these classes there is a marked decrease in k' when the size of the *ortho*-substituted alkyl groups increases. That alkyl groups in the *meta*- and *para*-positions also influence retention is clearly demonstrated by Fig. 2. To explain this behaviour we have to consider both the possible interaction between the aromatic ring and the stationary phase and the solubility in the mobile phase. It is reasonable to assume that when for compounds with the same *ortho*-substitution pattern the number of non-*ortho*-substituted alkyl groups increases, the aromatic ring will be less accessible for polar interaction with the cyano groups in the stationary phase. This effect will cause a decrease in k' values, *e.g.*, see 2,6-di-, 2,4,6-tri- and 2,3,5,6-tetra-methylphenols. At the same time, however, the solubility in the mobile phase will rise due to an increase in dispersion forces acting between the solute and the mobile phase. This second factor will also contribute to decreased retention.

It is of special interest to compare the retentions of 2,6-dimethylphenol (No. 7) and some 2-*tert*.-butylphenols (Nos. 10 and 11) on the silica and nitrile phase, respectively. On silica gel the latter phenols travel more rapidly than the former, while on the nitrile phase the reverse is true. This is in accordance with the difference in hydrogen-bond interactions for silanol and cyano groups, as has already been discussed.

Comparison of retentions and selectivity on conventionally coated and chemically bonded nitrile phases. For mono- and non-*ortho*-substituted phenols, the k' values in Table II are generally lower on Cyano Sil-X-I than on the conventionally coated phases, while for di-*ortho*-substituted phenols the retentions are similar or even greater on Cyano Sil-X-I (see Fig. 3a). The reason for this effect is considered to be mainly different mobile phase compositions and phase volume ratios. For the conventionally coated phases pure isooctane was used, while for Cyano Sil-X-I the mobile phase also

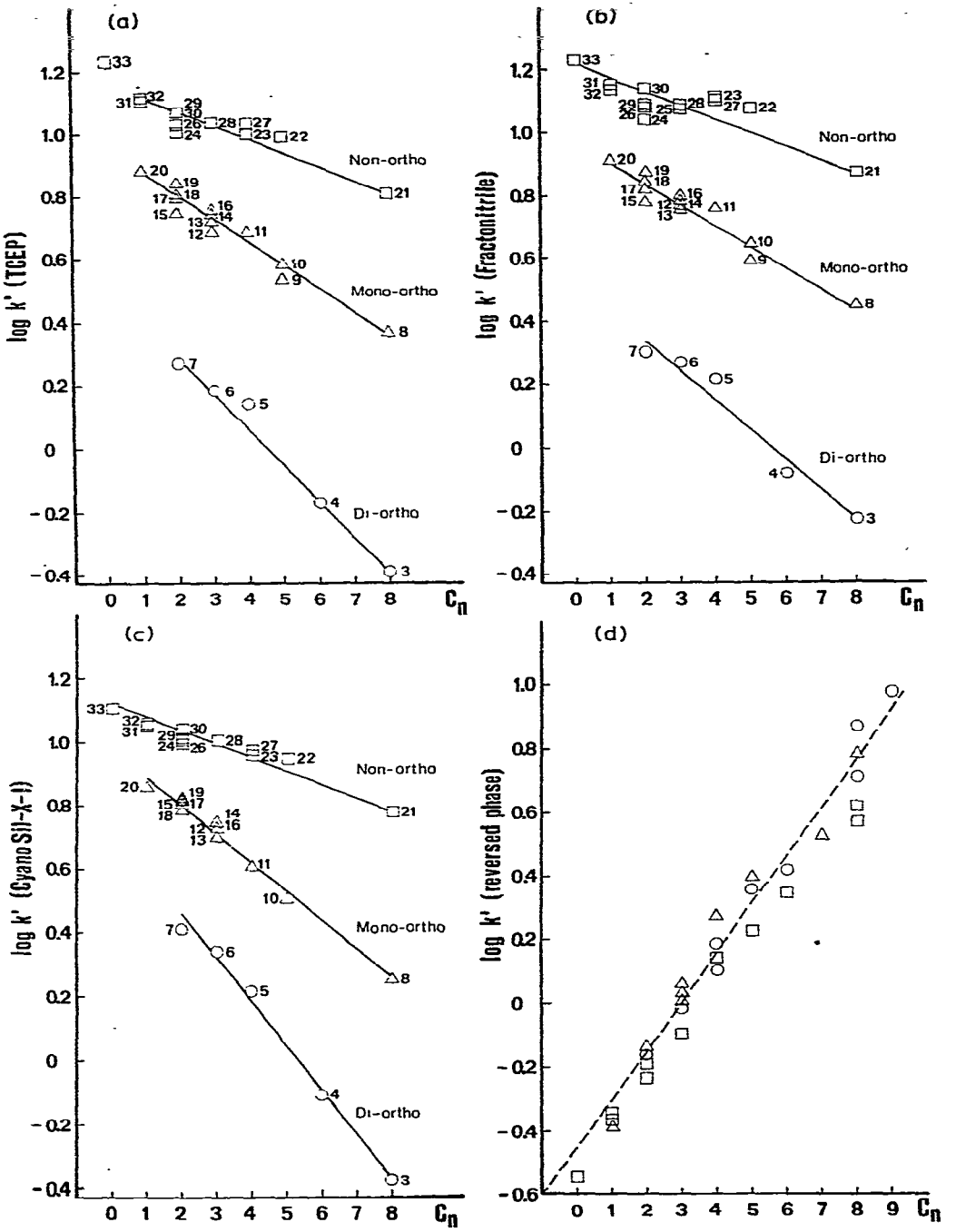


Fig. 2. Relationship between alkyl carbon number (C_n) and the retention of alkylphenols. (a) Stationary phase, TCEP; mobile phase, isooctane; 2.4 mm/sec. (b) Stationary phase, Fractonitril; mobile phase, isooctane; 2.4 mm/sec. (c) Stationary phase, Cyano Sil-X-I; mobile phase, 0.5% 2-propanol in isooctane; 2.5 mm/sec. (d) Stationary phase, μBondapak C_{18}; mobile phase, ethanol–water (60:40); 0.60 mm/sec. ○ = Di-*ortho*-substituted; △ = mono-*ortho*-substituted; □ = non-*ortho*-substituted alkylphenols.

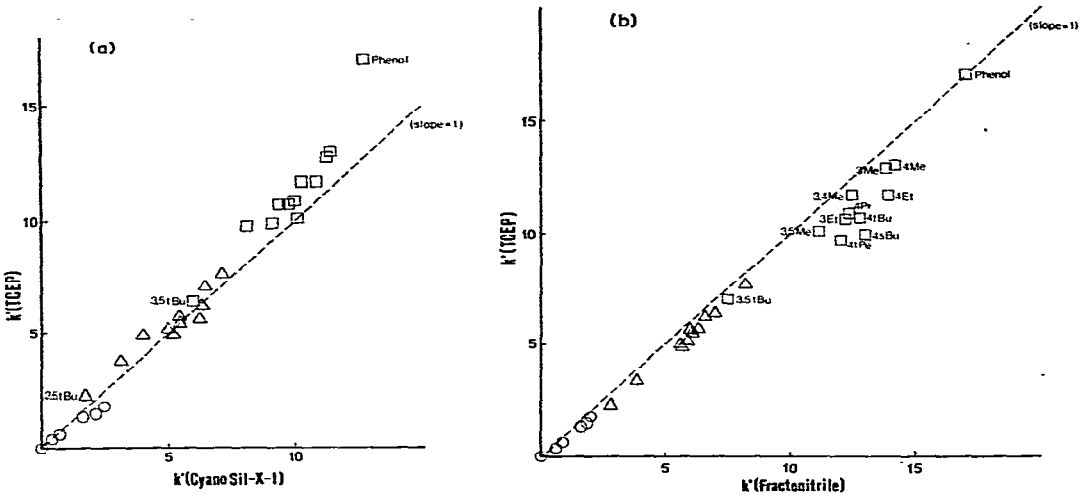


Fig. 3. Comparison of retentions of alkylphenols on different nitrile phases. (a) k' on TCEP–isooctane *versus* k' on Cyano Sil-X-I–0.5% 2-propanol in isooctane; (b) k' on TCEP–isooctane *versus* k' on Fractonitril–isooctane. ○ = Di-*ortho*-substituted; △ = mono-*ortho*-substituted; □ = non-*ortho*-substituted alkylphenols.

contained 0.5% (v/v) 2-propanol. Accordingly, in the latter instance hydrogen bonding to the mobile phase could contribute to a decrease in k', especially for the non-*ortho*-substituted phenols. For the two conventionally coated phases k' is generally higher for Fractonitril than for TCEP, as shown in Fig. 3b, which also illustrates that there is a tendency for 4-substituted phenols within the non-*ortho*-substituted class to be more retained on Fractonitril than on TCEP.

The variation in selectivity for certain pairs of phenols on the different nitrile columns is demonstrated in Fig. 4, which indicates that the contribution of adsorption due to surface silanol groups is higher on the chemically bonded phase than on

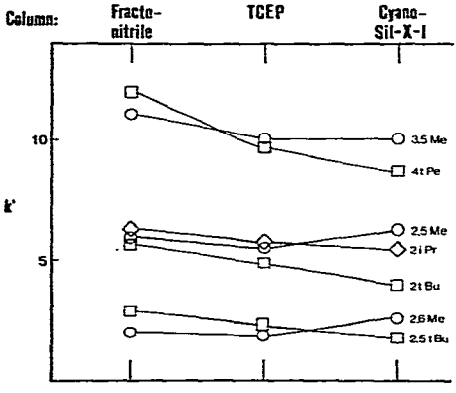


Fig. 4. Selectivity for some alkylphenols on Fractonitril–isooctane, TCEP–isooctane and Cyano Sil-X-I–0.5% 2-propanol in isooctane.

the conventionally coated phases, *i.e.*, the retention is more sensitive to the size of the *ortho*-substituent. This effect is further illustrated in Table III by the separation factors, α, of some alkylphenols for the TCEP, Cyano Sil-X-I and silica gel systems.

Reversed-phase LLC. In Table II are given the k' values for alkylphenols obtained in a reversed-phase system. The packing material was a microparticulate silica gel support with chemically bonded octadecylsilyl groups and the mobile phase was a mixture of ethanol and water.

TABLE III

SELECTIVITY COEFFICIENTS, α, FOR SOME ALKYLPHENOLS IN THE TCEP, CYANO SIL-X-I (FOR CONDITIONS, SEE TABLE I) AND THE TLC SYSTEM (SILICA GEL–BENZENE)

Pair of compounds	α		
	TCEP	*Cyano Sil-X-I*	SiO_2
2,6-Dimethyl–2-*tert.*-butyl-4-methyl	2.0	1.2	0.54
2,5-Dimethyl–2-isopropyl	1.02	0.87	0.74*
2,4-Dimethyl–2-ethyl	1.03	0.92	0.79

* Calculated from ref. 13.

Fig. 2d, in which log k' is plotted against the number of alkyl carbon atoms, shows that the retention increases with increasing number of carbon atoms, while there is no significant correlation between retention and the *ortho*-substitution pattern. This result is to be expected, considering the non-selective nature of the stationary phase.

The two main retention-determining factors in reversed-phase LC are considered to be dispersion forces acting between the stationary phase and the solute[24,32] and the solubility of the solute in the mobile phase[33,34,*]. For the non-*ortho*-substituted phenols the difference in solvation between individual compounds is less than for the two other classes because of the absenc of steric hindrance. It is observed that there is a steady increase in retention for 4-alkylphenols with the size of the alkyl group, which reflects the increase in dispersion forces between solute and stationary phase.

Comparison of retentions for the three structure classes shows that for phenols with one or two methyl groups, it is of minor importance for the magnitude of the retention whether the methyl groups are situated in the *meta*-, *ortho*- or *para*-positions. Thus, phenols with one methyl group have $k' = 0.42$–0.44 and for phenols with two methyl groups k' is 0.69 for di-*ortho*-substituted, 0.69 for mono-*ortho*-substituted and 0.57–0.64 for non-*ortho*-substituted phenols. However, with increasing size of the alkyl group, the retention difference between isomers increases. This effect is demonstrated in Table IV, where a comparison of the separation factors, α, for *ortho*- and *para*-isomers is made. Table IV also gives the k' and α values obtained by Bark and Graham[33] in a reversed-phase TLC study, using ethyl oleate on cellulose as the stationary phase and ethanol–water the mobile phase. The fact that *ortho*-isomers are eluted later than *para*-isomers for large alkyl groups can be ascribed to decreased solvation of the *ortho*-isomer due to steric hindrance. The influence of steric hindrance

* Recently the retention mechanism in reserved bonded phase chromatography has been evaluated in terms of the hydrophobic or solvophobic effect[35,36].

TABLE IV

RETENTION VALUES FOR 2- AND 4-SUBSTITUTED ISOMERS IN REVERSED-PHASE CHROMATOGRAPHY

Substituent, R	k' or k'_{TLC}		α
	o-R-C₆H₄OH	*p-R-C₆H₄OH*	
Methyl	0.44	0.42	1.00
Ethyl	0.74	0.66	1.12
n-Propyl	1.14	1.01	1.14
sec.-Butyl	—	1.36	—
tert.-Butyl	1.87	1.21	1.55
*Reversed-phase TLC**			
Methyl	0.27	0.20	1.35
Ethyl	0.64	0.43	1.49
n-Propyl	1.17	0.82	1.43
n-Butyl	—	1.67	—
sec.-Butyl	1.94	1.27	1.53
tert.-Butyl	3.17	1.08	2.94

* Values calculated from ref. 33.

on solvation is further illustrated by the k' values for 2,6-, 2,5- and 3,5-di-*tert.*-butylphenols of 7.4, 6.0 and 3.7, respectively.

The effect of alkyl chain branching on retention has been observed for isomeric alcohols in reversed bonded phase chromatography[24]. This effect has been studied further by chromatographing some C_1–C_5 aliphatic alcohols on μBondapak C_{18} with methanol–water (5:95) as the mobile phase. In Fig. 5, log k' for the alcohols is

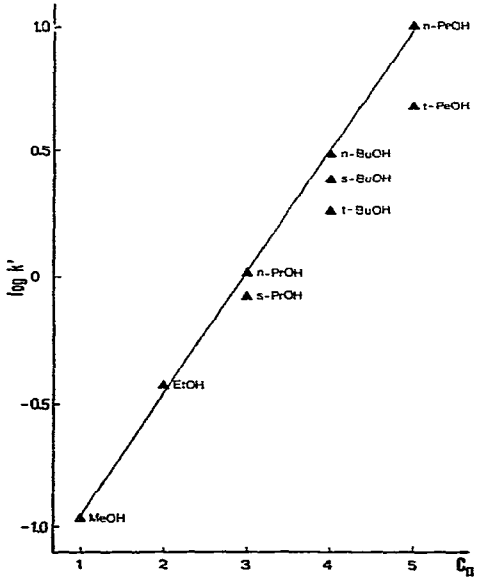


Fig. 5. Effect of branching on retention of aliphatic alcohols. Stationary phase, μBondapak C_{18}; mobile phase, methanol–water (5:95); 0.6 ml/sec.

plotted against carbon number. As can be seen for the butyl alcohols, for instance, *tert.*-butyl alcohol is eluted first followed by the secondary and normal alcohols. The same branching effect is observed for 4-*sec.*-butyl- and 4-*tert.*-butylphenol, the latter being eluted first (see Table IV). Comparison with the k' values of 2,6-di-*tert.*-butyl- and 2,6-di-*sec.*-butylphenol in Table II shows that in this instance the order of elution is reversed, indicating that the branching effect is cancelled by the greater steric hindrance of the *tert.*-butyl groups. The same effect is observed for 2-*tert.*-butyl- and 2-*sec.*-butylphenol (see the k'_{TLC} values of Bark and Graham[33] in Table IV).

Application to analysis

Combination of straight- and reversed-phase LLC. In Fig. 6, log k' for the reversed-phase LC system is plotted against log k' for a straight-phase system, Cyano Sil-X-I. It can be seen that all of the phenols investigated are clearly divided into the three structure classes. Accordingly, it appears to be possible to recognize di-, mono- and non-*ortho*-substituted phenols by combining straight- and reversed-phase chromatography. Another characteristic of the two column plots in Fig. 6 is that within each structure group the points are arranged according to alkyl carbon number. Thus, starting from the top of each structure line, the alkyl carbon number will decrease along the line. Points that represent phenols with the same alkyl carbon number tend to cluster, which makes identification of individual phenols difficult. For a complete separation of these phenols, we used capillary column GC as described below. However, it should be pointed out that it ought to be possible to achieve a better resolution of the points in Fig. 6 by optimizing the composition of the mobile phase for certain phenols.

In Fig. 7, the separation of a nine-component mixture of alkylphenols by combining straight- and reversed-phase LC is demonstrated. The first chromatogram (Fig. 7a) shows the separation and fraction collection (I–III) on the straight-phase system and Fig. 7b, c and d show the chromatograms of the fractions I, II and III,

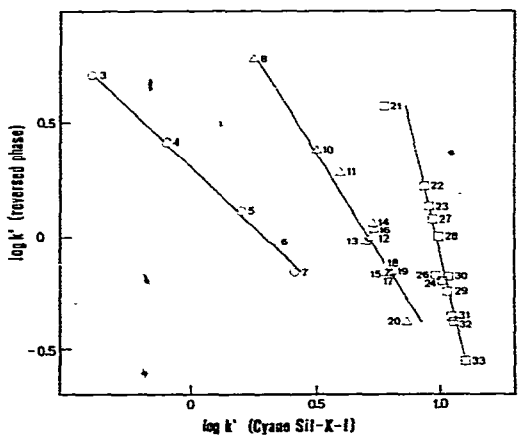


Fig. 6. Two-column plot of log k' on reversed phase [μBondapak C_{18}–ethanol–water (60:40)] *versus* log k' on straight phase (Cyano Sil-X-I–0.5% 2-propanol in isooctane). ○ = Di-*ortho*-substituted; △ = mono-*ortho*-substituted; □ = non-*ortho*-substituted. Numbers of alkylphenols refer to Table II.

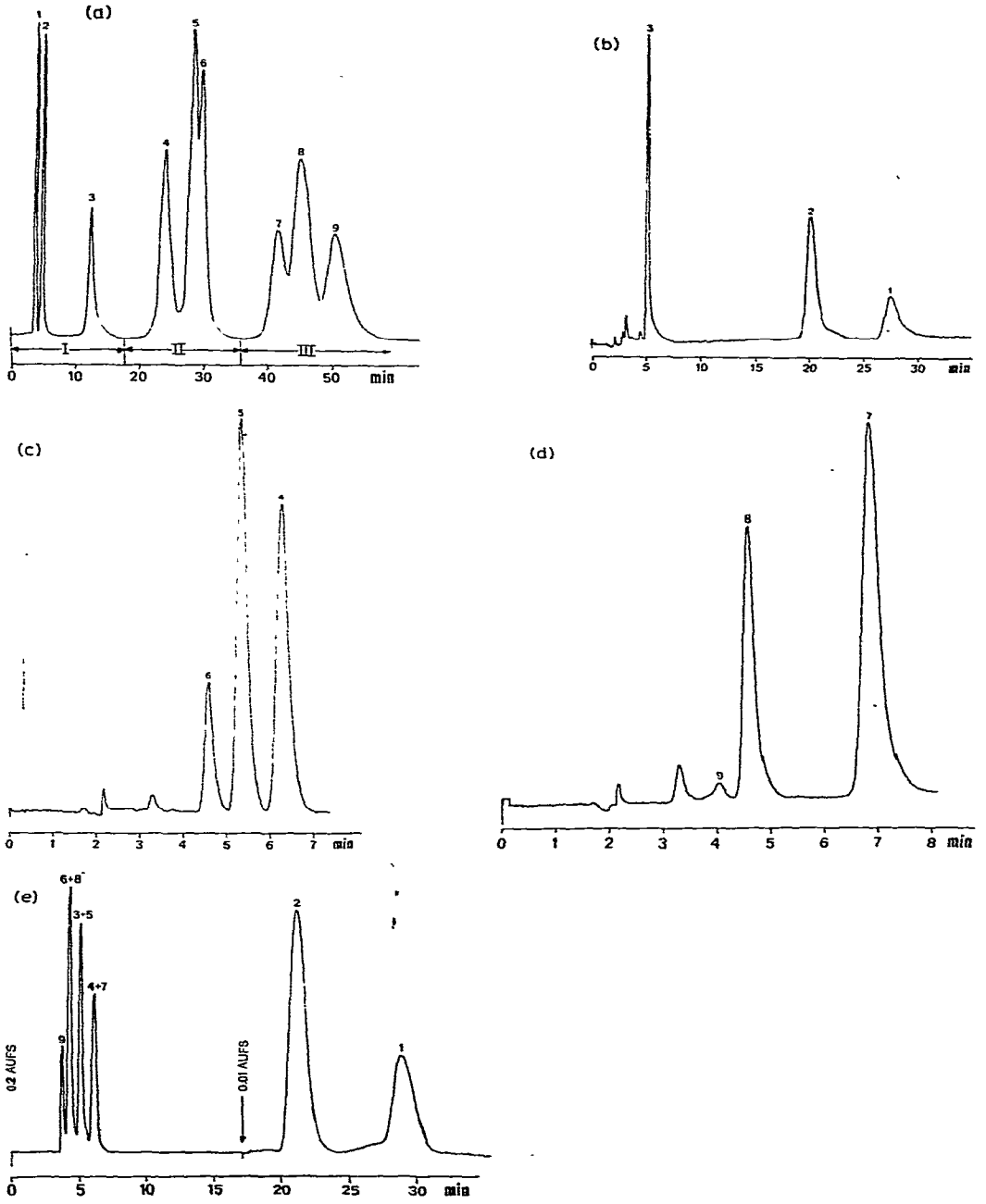


Fig. 7. Separation of a nine-component mixture by combining straight- and reversed-phase liquid chromatography. (a) Fraction collection (I–III) on Cyano Sil-X-I column. Mobile phase, 0.5% 2-propanol in isooctane; 2.5 mm/sec. (b) Fraction I from (a) chromatographed on μBondapak C_{18}–ethanol–water (60:40); 1.8 mm/sec. (c) Fraction II from (a) chromatographed on μBondapak C_{18}–ethanol–water (60:40); 1.8 mm/sec. (d) Fraction III from (a) chromatographed on μBondapak C_{18}–ethanol–water (60:40); 1.8 mm/sec. (e) All nine alkylphenols chromatographed on μBondapak C_{18}–ethanol–water (60:40); 1.8 mm/sec. Peak identity: 1 = 2,6-di-*tert.*-butyl; 2 = 2,6-di-*sec.*-butyl; 3 = 2,6-dimethyl; 4 = 2,3,5-trimethyl; 5 = 2,4-dimethyl; 6 = 2-methyl; 7 = 4-propyl; 8 = 4-methyl; 9 = phenol.

respectively, on the reversed-phase system. For comparison, the chromatogram from the separation of all nine phenols on the reversed-phase system is also given (Fig. 7e).

Application of gas chromatography. As already mentioned, GLC has been the preferred method for the separation of mixtures of alkylphenols. The various GLC methods that have been applied can be divided into the following groups; (i) GLC of free phenols on polar stationary phases on packed columns[1–3], on open-tubular columns[4,5] or on packed capillary columns[6]; (ii) GLSC (gas–liquid–solid chromatography) of free phenols on packed columns[7,8]; and (iii) GLC of phenol derivatives on packed columns or on open-tubular columns[9].

GLC of free alkylphenols on packed columns[1–3] was the first and most frequently used gas chromatographic method. Much of the work has dealt with the separation and quantitation of phenol, cresols and xylenols, which are the main phenolic components in the lower boiling fractions of coal tar. The chief separation problem arises from the difficulty of resolving *m*- and *p*-cresol, the corresponding ethylphenols and 2,4- and 2,5-xylenol. This task has been accomplished by the use of selective stationary phases, generally various kinds of alkyl phthalates and aryl phosphates[2,3]. The resolution is improved by the introduction of open-tubular columns. For instance, Ettre[4] describes a separation of a mixture of phenol and 15 C_1–C_3-alkylphenols using an open-tubular column coated with didecyl phthalate and Hrivnák and Macák[5] studied the application of various alkyl phthalates and aryl phosphates on open-tubular columns to the analysis of a 10-component mixture of phenol and C_1–C_2-methylphenols. The best separation was obtained on tri(2,4-xylenyl)-phosphate.

Landault and Guiochon[6] used a packed capillary column coated with a mixture of cyclohexyl phthalate and xylenyl phosphate for the separation of a 20-component mixture of phenol and C_1–C_5-methylphenols. A different approach to the actual separation problem was presented by Di Corcia[7], who used graphitized carbon black (Carbopack) as the support, coated with a low percentage of a stationary phase (GLSC).

A very high separation efficiency was achieved with a method described by Grant and Vaughan[9], who made an attempt to separate a mixture containing most of the C_3–C_4 alkylphenols found in coal tar, including tri- and tetramethylphenols, methylethylphenols, diethylphenols, propylphenols, methylpropylphenols, butylphenols and indanols. They succeeded in resolving a mixture of about 60 phenols as their trimethylsilyl (TMS) ethers into 45 peaks using a glass capillary column coated with silicone oil.

For very complex mixtures of alkylphenols, the resolution attainable, even in long open-tubular columns, is not sufficient for a complete separation. A pre-separation of the phenolic mixture could help to solve this problem. As already discussed, information about the type of *ortho*-substitution and alkyl carbon number can be obtained by LC. By subjecting groups of phenols isolated by LC to capillary column GC, the possibility of separating complex phenolic mixtures into their components would increase considerably.

The choice between GC methods is open for discussion and is to some extent related to the composition of the phenolic mixture. Although the application of a strongly polar stationary phase such as TCEP opens up certain possibilities for the separation of phenols belonging to the same structure group and with the same alkyl carbon number (see Fig. 8), we have chosen a GC procedure similar to that described

by Grant and Vaughan[9]. The main reason is the difficulties encountered when analyzing free phenols on open-tubular columns coated with a strongly polar liquid. Fig. 8 also demonstrates the inability of GC to separate alkylphenols according to *ortho*-substitution as compared with the retention behaviour on the same stationary phase (TCEP) in LC (*cf.* Fig. 2a).

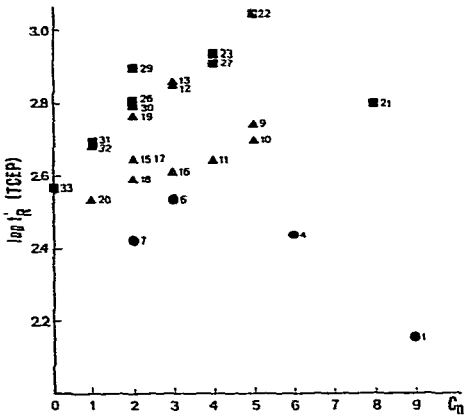


Fig. 8. Relationship between alkyl carbon number, C_n, and retention for alkylphenols in gas chromatography on TCEP as stationary phase. Column: 5% TCEP on Chromosorb G HP 80–100 mesh, 1.8 m × 2 mm. Temperature, 160°; nitrogen flow-rate, 30 ml/min. Numbers refer to compounds in Table V.

The method used for the separation of alkylphenols by GC involved derivatization to TMS ethers, which were chromatographed on an open-tubular column coated with Apiezon L. The retention values of the silylated phenols are summarized in Table V, and in Fig. 9 the separation obtained for the non-*ortho*-substituted group of alkylphenols is demonstrated. It can be seen that the 13-component mixture gives 13 peaks, although the pairs 3-ethyl-phenol–3,5-dimethylphenol and 4-*tert.*-pentylphenol–3,5-di-*tert.*-butylphenol are only partly resolved. The mono-*ortho*-substituted group of alkylphenols can be wholly or partly resolved when chromatographed as TMS ethers on Apiezon L, with the exception of the pair 2-propylphenol–2,3-dimethylphenol, and all phenols in the di-*ortho*-substituted groups are wholly resolved after silylation. Two well separated peaks were obtained when chromatographing the TMS ether of 2,6-di-*sec.*-butylphenol, which are due to the racemate and the meso-form, respectively. A chromatogram is shown in Fig. 10.

Accordingly, only two of the 34 phenols remain completely unresolved if the alkylphenols are first separated into structure groups by LC. However, 2-propyl- and 2,3-dimethylphenol can be separated on the reversed-phase LC system as they belong to different carbon number groups. The result should be compared with that which would have been obtained if TMS ethers of all 34 phenols investigated had been chromatographed together on Apiezon L. Comparison between the retention values given in Table V shows that five unresolved peaks, each containing two or more phenols, would have resulted.

TABLE V

CORRECTED RETENTION TIMES IN GAS CHROMATOGRAPHY FOR ALKYLPHENOLS ON A PACKED TCEP COLUMN AND FOR THE CORRESPONDING TMS ETHERS ON AN OPEN-TUBULAR APIEZON L COLUMN

No.	*Compound*	*Corrected retention time (sec.)*	
		TCEP	*Apiezon L**
	Di-ortho-substituted		
1	2,6-Di-*tert.*-butyl-4-methyl	140	2160
2	2,6-Di-*tert.*-butyl	—	1760
3	2,6-Di-*sec.*-butyl	—	875, 914**
4	2,6-Di-isopropyl	270	412
5	2,3,5,6-Tetramethyl	—	806
6	2,4,6-Trimethyl	350	395
7	2,6-Dimethyl	260	235
	Mono-ortho-substituted		
8	2,5-Di-*tert.*-butyl	—	920
9	2-*tert.*-Butyl-5-methyl	550	735
10	2-*tert.*-Butyl-4-methyl	500	466
11	2-*tert.*-Butyl	440	313
12	2,4,5-Trimethyl	710	348
13	2,3,5-Trimethyl	720	339
14	2-Propyl	—	231
15	2.5-Dimethyl	440	173
16	2-Isopropyl	410	190
17	2,4-Dimethyl	440	204
18	2-Ethyl	390	162
19	2,3-Dimethyl	580	232
20	2-Methyl	340	116
	Non-ortho-substituted		
21	3,5-Di-*tert.*-butyl	630	654
22	4-*tert.*-Pentyl	1100	627
23	4-*sec.*-Butyl	850	404
24	3,5-Dimethyl	—	186
25	3,4,5-Trimethyl	—	449
26	3-Ethyl	640	178
27	4-*tert.*-Butyl	800	370
28	4-Propyl	—	326
29	3,4-Dimethyl	790	243
30	4-Ethyl	610	202
31	3-Methyl	490	117
32	4-Methyl	480	128
33	Phenol	371	73

* Retention time for the corresponding TMS ether.

** The two retention values given refer to the racemate and mesoform, respectively.

CONCLUSION

In LC, the correlation between retention and number of *ortho*-substituted groups for alkylphenols is better on nitrile phases than on silica gel, which can be explained by the difference in nature of the hydrogen-bonding mechanism. The combination of straight- and reversed-phase LC can be used for the separation of complex

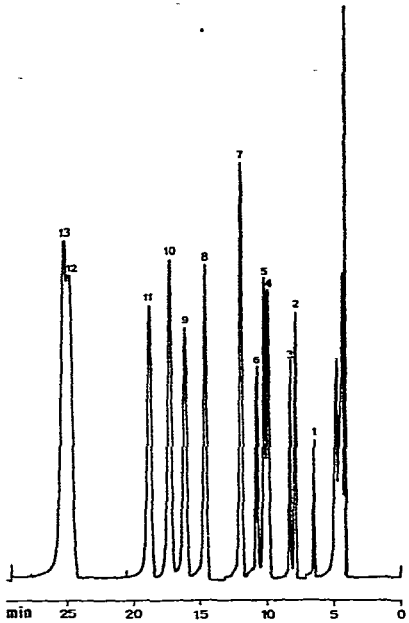


Fig. 9. Separation of silylated non-*ortho*-substituted alkylphenols on a 150-ft. capillary GC column. Stationary phase, Apiezon L; temperature, 140°; nitrogen flow-rate, 2 ml/min. Peak identity: 1 = phenol; 2 = 3-methyl; 3 = 4-methyl; 4 = 3-ethyl; 5 = 3,5-dimethyl; 6 = 4-ethyl; 7 = 3,4-dimethyl; 8 = 4-propyl; 9 = 4-*tert.*-butyl; 10 = 4-*sec.*-butyl; 11 = 3,4,5-trimethyl; 12 = 4-*tert.*-pentyl; 13 = 3,5-di-*tert.*-butyl.

Fig. 10. Separation of the racemate and the mesoform of silylated 2,6-di-*sec.*-butylphenol. Column, capillary Apiezon L, 150 ft.; temperature, 140°; flow-rate, 2 ml/min.

mixtures of phenols and for the identification of the individual compounds as regards *ortho*-substitution pattern and alkyl carbon number. The combination of LC and capillary column GC presented provides an effective means for the separation of very complex mixtures of alkylphenols.

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