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# LIQUID CHROMATOGRAPHIC STUDY OF BROMINATED ALKYLPHENOLS AND INVESTIGATION OF PRODUCT FORMATION IN THE COULOMETRIC BROMINATION OF SOME ALKYL-SUBSTITUTED PHENOLS

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## SUMMARY

Straight- and reversed-phase liquid chromatography (LC) have been used to study product formation in the coulometric bromination of phenol, 4-alkylphenols and 2,4- and 2,6-dialkyl-substituted phenols. The coulometric method yields predictable, unambiguous products by exchange of hydrogen for bromine in free *ortho*- and *para*-positions. The introduction of bromine into an alkylphenol results in an increase in retention on the reversed-phase LC system and there is a linear correlation between the capacity factors for the brominated and non-brominated phenols. In the straight-phase LC system the introduction of bromine into the *ortho*-positions of 2,4- and 4-alkylphenols results in a marked decrease in retention, while *para*-substitution of 2,6-alkylphenols causes an increase. Combination of LC and coulometric bromination can be used for the separation of isomeric alkylphenols, *e.g.*, 3- and 4-alkylphenols.

## INTRODUCTION

In a previous paper[1] it was shown that alkylphenols can be quantitatively brominated with a coulometric technique based on reaction with anodically generated bromine. The advantage of that method compared with other methods based mainly on volumetric bromination[2] is that the reaction can be controlled by means of the titration medium and by using an optimal generating current. The method can therefore be applied to a large number of phenols, even those which are usually sensitive to side-reactions.

The aim of this investigation was to study product formation by liquid chromatography (LC) at various stages during the coulometric bromination and at the same time to examine the retention behaviour of brominated alkylphenols in different LC systems. In this work the choice of alkylphenols has been restricted to some species which, on monobromination, can form only one unambiguous bromophenol, *viz.*, 4-alkylphenols and 2,4- and 2,6-dialkylphenols. In addition, the bromination products of phenol and 2,3,5,6-tetramethylphenol have been investigated in some detail.

On monobromination, the products expected to be formed from 4-alkyl- and

2,4- and 2,6-dialkylphenols are 2-bromo-4-alkyl-, 6-bromo-2,4-dialkyl- and 4-bromo-2,6-dialkylphenols, respectively[3]. 4-Alkylphenols can also be fully brominated to give 2,6-dibromo-4-alkylphenols. In a forthcoming paper the course of the bromination of alkylphenols which can yield more than one regular monobromination product, *e.g.*, 2- and 3-alkylphenols, will be examined.

An example is also given in this paper which demonstrates the possibility of using coulometric bromination in conjunction with LC for the resolution of phenolic mixtures which are difficult to separate as such by chromatographic methods, *e.g.*, 3- and 4-alkylphenols.

## EXPERIMENTAL

### *Apparatus*

*Coulometric titration.* The apparatus and procedure for the coulometric bromination have been described in detail elsewhere[1].

*Liquid chromatography.* Two pumps were used, namely a Varian 4100 positive displacement pump (Varian, Walnut Creek, Calif., U.S.A.) and a Milton Roy mini-pump equipped with a pulse dampener (LDC-709, Laboratory Data Control, Riviera Beach, Fla., U.S.A.). The detectors used were an LDC 1285 UV detector (280 or 254 nm) and a single-beam detector with variable wavelength, 190–700 nm (LC-55, Perkin-Elmer, Norwalk, Conn., U.S.A.). Sample applications were effected either by syringe injection or with a valve injector (Rheodyne, Berkeley, Calif., U.S.A.).

*Columns.* The columns were either commercially available pre-packed micro-particulate columns ($\mu$Bondapak $C_{18}$, 4 mm I.D. $\times$ 300 mm, Waters Assoc., Milford, Mass., U.S.A., and Cyano Sil-X-I, 2.6 mm I.D. $\times$ 500 mm, Perkin-Elmer) or dry-packed with Corasil $C_{18}$ (Waters Assoc.) in precision-bore stainless-steel tubing (2.1 $\times$ 500 mm) by the tap-and-fill method.

### *Chemicals*

Isooctane (certified A.C.S. grade, Fisher Scientific, Fairlawn, N.J., U.S.A.), methanol (analytical-reagent grade, May & Baker, Dagenham, Great Britain), ethanol (absolute, 99.5%, Kemetylprodukter AB, Bromma, Sweden) and 2-propanol (pro analysi grade, E. Merck, Darmstadt, G.F.R.) were used as the liquid chromatographic eluents.

Acetic acid (pro analysi grade, Merck), pyridine (analytical-reagent grade, Mallinckrodt, New York, N.Y., U.S.A.) and sodium bromide (99%, BDH Chemicals, Poole, Great Britain) were used for the preparation of the titration media. Methylene chloride (99%, Kebo AB, Stockholm, Sweden) was used for the extraction procedure.

All phenols used were of the best quality commercially available and in some instances were purified by recrystallization or distillation. All other chemicals were used without further purification.

### *Procedure*

Alkylphenols, 10–20 $\mu$equiv. in 20 ml of titration medium, were coulometrically brominated as described by Kinberger *et al.*[1]. Most of the phenols were monobrominated in medium III-2 (60%, v/v, acetic acid, 40% water and a bromide concentration of 0.4 mole$\cdot$l$^{-1}$). Full bromination was performed in medium II-2 (55%, v/v,

acetic acid, 40% water, 5% pyridine and a bromide concentration of 0.4 mole·$l^{-1}$). Samples (5–100 $\mu$l) containing 1–8 nmole were removed at different stages of the titration directly from the titration vessel by means of a micro-syringe after stopping the generating current. The sample was generally injected on to the LC column but for the Corasil $C_{18}$ column an extraction procedure was included in order to remove the pyridine.

*Extraction procedure.* The titration mixture (20 ml) was transferred into a 50-ml separating funnel, 20 ml of water were added and the mixture was extracted with three 1.5-ml volumes of methylene chloride. The organic layer was washed with three 1.5-ml volumes of dilute hydrochloric acid (5 mole·$l^{-1}$) and was transferred into a graded centrifuge tube. The solvent was evaporated with a gentle flow of nitrogen to a volume of about 100 $\mu$l and 3–5 $\mu$l were injected on to the LC column.

## RESULTS AND DISCUSSION

### *Choice of liquid chromatographic systems*

It has been shown that the combination of straight- and reversed-phase liquid chromatography can give valuable information about the structure of alkylphenols[4]. On a straight-phase nitrile system it is the substitution in the *ortho*-positions that determines the retention, while in the reversed-phase system it is primarily the number of alkyl carbon atoms that affects the retention.

The same types of chromatographic systems have been used in this work, as follows: one straight-phase system, a chemically bonded nitrile phase (Cyano Sil-X-I) with isooctane + 0.5%, v/v, of 2-propanol as the mobile phase, and two reversed-phase systems, one pellicular (Corasil $C_{18}$) and one microparticulate ($\mu$Bondapak $C_{18}$) octadecylsilane phase with water–alcohol as the mobile phase. The capacity factor, $k'$, can be controlled by varying the alcohol content of the mobile phase, as illustrated in Fig. 1. About the same selectivity was obtained for the two reversed-phase systems. The separation factor, $\alpha$, between the solutes in Fig. 1 is little influenced by the

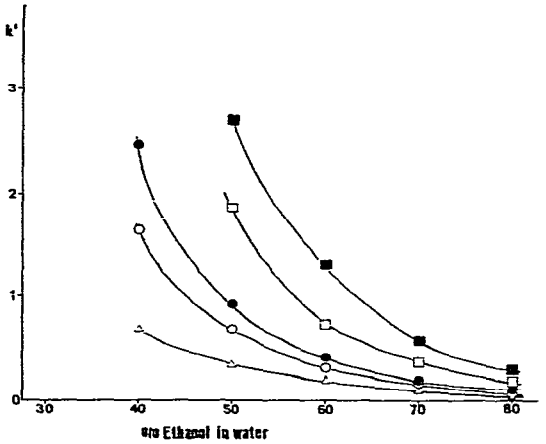
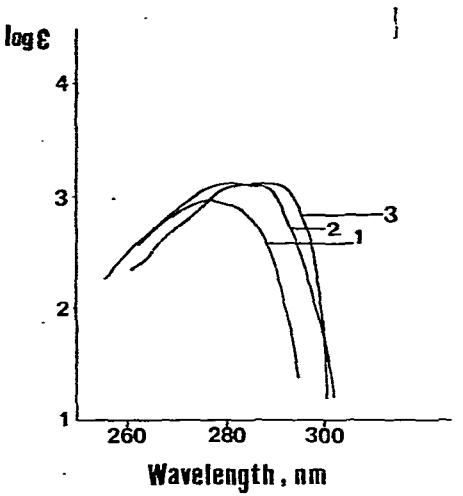



Fig. 1. Relationship between capacity factor, $k'$, for phenol and bromophenols and ethanol content of the mobile phase. Column: $\mu$Bondapak $C_{18}$. Eluent: ethanol–water. Flow-rate: 20 ml·$h^{-1}$. △, Phenol; ○, 2-bromophenol; ●, 4-bromophenol; □, 2,4-dibromophenol; ■, 2,4,6-tribromophenol.



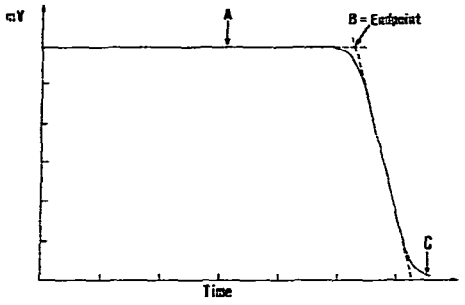




Fig. 2. UV spectra of 4-methylphenol and its bromination products. 1 = 4-Methylphenol; 2 = 2-bromo-4-methylphenol; 3 = 2,6-dibromo-4-methylphenol.

Fig. 3. Titration curve for coulometric bromination of alkylphenols. A, B and C indicate points at which samples were removed from the titration vessel.

alcohol concentration. In order to obtain reasonable elution times and satisfactory separation, the composition ethanol–water (60:40) was generally chosen for the microparticulate column and methanol–water (40:60) for the pellicular column. The retention of the phenols on Cyano Sil-X-I depends very much on the concentration of 2-propanol in the mobile phase so that careful preparation of the mobile phase is necessary. The separation factors on Cyano Sil-X-I for phenol and some brominated phenols are shown in Table I.

*Detection wavelengths.* The detection wavelength was 280 nm. However, on full bromination the sample contains pyridine, which can interfere with early eluting peaks when detection is carried out at this wavelength. This difficulty can be avoided by operating the detector at 290 nm instead. For alkylphenols, this change decreases the sensitivity considerably while, for *ortho*-brominated phenols, the change in sensitivity is negligible owing to the bathochromic shift that occurs when bromine is introduced at *ortho*-positions in an alkylphenol, as shown in Fig. 2.

*Introduction of sample.* On the reversed-phase microparticulate column, direct

TABLE I

SEPARATION FACTORS, $\alpha$, FOR PHENOL AND BROMOPHENOLS IN THE STRAIGHT-PHASE LC SYSTEM

Column: Cyano Sil-X-I. Eluent: 0.5% (v/v) 2-propanol in isooctane.

| *Pair of phenols* | $\alpha$ | $k'^*$ |
|---|---|---|
| Phenol/2-bromophenol | 3.69 | 9.53 |
| 4-Bromophenol/2-bromophenol | 4.78 | 12.33 |
| 2,4-Dibromophenol/2-bromopheno | 1.82 | 4.70 |
| 2,4-Dibromophenol/2,4,6-tribromophenol | 3.03 | 4.70 |

* For the most retained solute.

injection of the sample was carried out without any decrease in column performance. However, with the pellicular reversed-phase column it was necessary to remove pyridine, if present, by an extraction procedure in order to prevent interference. Direct injection was also performed on the straight-phase system but this procedure had certain disadvantages. For example, a slight baseline disturbance was observed and a gradual decrease of about 10% in the retention of the reference substance, 2,6-dimethylphenol, during the course of this work, was believed to be due to the acetic acid in the injected sample, the concentration of which was always *ca.* 60%.

*Product formation in coulometric bromination*

In the coulometric method for the bromination of alkylphenols described by Kinberger *et al.*[1], the reaction is carried out in a water–acetic acid medium and the reactivity is controlled by varying the water content, the bromide ion concentration and by the addition of pyridine. The reaction is believed to involve substitution with bromine at the free *ortho*- and *para*-positions[3]. For phenols with more than one free *ortho*- and *para*-positions the titration can be carried out to either the monobrominated or the fully brominated stage, depending on the "reactivity" of the titration medium. The titration is followed by means of a dead-stop indication technique[5] and a typical titration curve is shown in Fig. 3. The product formation during the titration was followed by removing samples from the titration vessel at different stages of the titration as indicated (A, B and C) in Fig. 3. The samples were injected directly on to the μBondapak column. An example is given by the chromatograms in Fig. 4, which represent the monobromination of phenol. The chromatograms show the decrease in the phenol peak while the peaks representing the monobrominated products increase (*cf.*, A and B). At the end-point (B), the reaction mixture contains phenol and 2-bromo- and 4-bromophenol. After the end-point (C), all phenol has been consumed and dibrominated products begin to appear.

The identities of the peaks were confirmed by comparison with chromatograms obtained for reference substances of bromophenols on the reversed-phase system. In the same way, 4-methyl- and 2,6-dimethylphenol were shown to yield only 2-bromo-4-

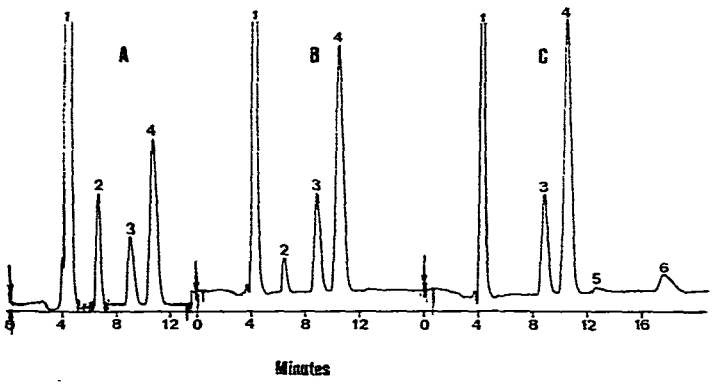



Fig. 4. Chromatograms of the product mixture after monobromination of phenol. Column: μBondapak $C_{18}$. Eluent: ethanol–water (50:50, v/v). Flow-rate: 40 ml·$h^{-1}$. Wavelength: 280 nm. Volume injected: 50 μl. Peaks: 1 = acetic acid; 2 = phenol; 3 = 2-bromophenol; 4 = 4-bromophenol; 5 = 2,6-dibromophenol; 6 = 2,4-dibromophenol.

methyl- and 4-bromo-2,6-dimethylphenol, respectively, when titrated with bromine to the end-point using monobromination conditions. Full bromination of 4-methylphenol produced 2,6-dibromo-4-methylphenol, when the sample was taken at the end-point.

Analogous results were obtained on bromination of other 4-alkyl- and 2,6-dialkylphenols. Thus, when samples were taken at the end-point only one main peak appeared in the chromatogram. On monobromination, a small peak due to unreacted phenol was observed in some instances, especially for 2,6-dialkylphenols, which explains their tendency to give low results in quantitative determinations[1]. Although no reference substances for the bromophenols were available for comparison, the structure was confirmed by the retention pattern in straight- and reversed-phase LC (Figs. 7 and 8) and by the linear relationships found between the k' values for brominated and non-brominated alkylphenols (Fig. 10).

For the bromination products of 2,4-dialkylphenols, no comparison has been made with reference substances but the products are likely to be 6-bromo-2,4-dialkylphenols. Only one product was observed at the end-point and the retention patterns in straight- and reversed-phase LC confirmed that this was the expected bromophenol (Fig. 9).

*Effect of over-bromination.* As described in the previous section, coulometric titration of the alkylphenols up to the end-point and in the correct titration medium proceeds with the formation of *ortho*- and *para*-bromophenols owing to exchange of hydrogen for bromine at free *ortho*- and *para*-positions. This result shows that the conditions recommended by Kinberger *et al.*[1] favour the nuclear substitution reaction and give an unambiguous product with negligible side-reaction, a fact which is reflected in the good quantitative results obtained with the method.

It is well known that in the analysis of alkylphenols by bromination, some phenols are able to consume more bromine than the stoichiometric amount corresponding to the free *ortho*- and *para*-positions[2]. Examples of side-reactions that have been suggested or proved to take place are bromination in *ortho*- and *para*-situated alkyl groups[3] or replacement of such groups with bromine[6]. The formation of bromocyclohexadienones has also been described in the literature[7,8]. This reaction has been utilized for the analysis of 2,4,6-trimethylphenol by coulometric bromination[1].

In order to examine the sensitivity of the phenols to an excess of bromine, samples were removed after an excess of 30% of bromine had been generated (point C in Fig. 3) and investigated with the reversed-phase LC system. On over-bromination of phenols with more than one free *ortho*- and *para*-position, using monobromination conditions, the nuclear substitution proceeds with the formation of dibromophenols, as shown for phenol in Fig. 4. Similarly over-brominated 4-alkylphenols yielded 2,6-dibromo-4-alkylphenols in addition to the monobrominated product, which was the only product observed at the end-point.

For phenols with only one free *ortho*- and *para*-position, the further reaction at over-bromination can take another course. Thus, in the chromatogram of over-brominated 2,6-dimethylphenol a new peak appears close to the front (see Fig. 5), which is assumed to be due to the formation of 2,6-dimethyl-4,4-dibromo-2,5-cyclohexadienone. This assumption was supported by the isolation and identification of the corresponding compound formed by a vigorous volumetric over-bromination of 4-methylphenol. The structure of the compound was investigated by means of nuclear

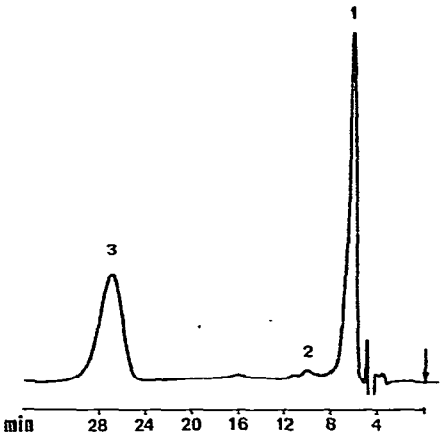
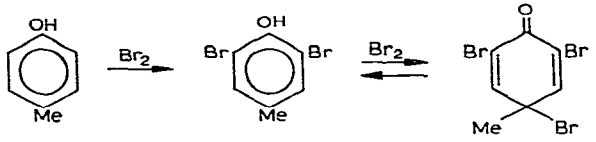



Fig. 5. Chromatogram of the product mixture after over-bromination of 2,6-dimethylphenol. Column: Corasil $C_{18}$. Eluent: methanol–water (40:60, v/v). Flow-rate: 25 ml·h$^{-1}$. Wavelength: 280 nm. Volume injected: 5 $\mu$l. Peaks: 1 = 4,4-dibromo-2,6-dimethyl-2,5-cyclohexadienone; 2 = 2,6-dimethylphenol; 3 = 4-bromo-2,6-dimethylphenol.

magnetic resonance, infrared and ultraviolet spectrometry, gas chromatography–mass spectrometry and elemental analysis, which indicated that the following reaction occurred:




The last step in the reaction is reversible and, on addition of thiosulphate, the cyclohexadienone is converted into 2,6-dibromo-4-methylphenol. Other 2,6-dialkylphenols tested did not form quinoid products on coulometric over-bromination. As the molar absorptivity of the bromocyclohexadienones is about ten times greater than that for the corresponding phenol[9], the relative amount of the compounds as visualized in the chromatogram is misleading.

Of the 2,4-dialkylphenols investigated, 2-*tert.*-butyl-4-methylphenol was the only one that showed any tendency to form by-products on over-bromination. Thus, three new peaks appeared in the chromatogram in addition to that for 6-bromo-2-*tert.*-butyl-4-methylphenol, which was the only product observed at the end-point. An early eluting peak is probably due to a bromocyclohexadienone, and the other two have retentions corresponding to 2-bromo- and 2,6-dibromo-4-methylphenol. These bromophenols can be formed by replacement of the *tert.*-butyl group with bromine to give 2-bromo-4-methylphenol, which is then further brominated to the dibromophenol[6].

It was previously mentioned that 2,4,6-trimethylphenol, which has no vacant *ortho*- or *para*-positions, yields quantitative results on coulometric bromination. The reaction takes place under full bromination conditions and is analogous to that of over-bromination of 2,6-dimethylphenol, *i.e.*, a *p*-quinoid bromocyclohexadienone is

formed[1,8]. It is of interest to compare the reactivity of this 2,4,6-trialkylphenol with that of other 2,4,6-trialkylphenols. Thus, it was shown that 2,6-di-*tert.*-butyl-4-methylphenol did react to a certain extent, while 2,4,6-tri-*tert.*-butylphenol did not[1].

*Influence of titration medium on product formation.* It has been shown that the composition of the titration medium has a decisive influence on the bromination reaction, which is reflected in the shape of the titration curve[1]. If a medium is used that promotes bromination too strongly, the risk of side-reactions occurring is high. Some phenols are more sensitive to the choice of titration medium than others. An example is given by the titration of 2,3,5,6-tetramethylphenol in two different media (Fig. 6). In the first chromatogram (Fig. 6a) 2,3,5,6-tetramethylphenol has been titrated in the recommended medium and, as can be seen, only one product (2,3,5,6-tetramethyl-4-bromophenol) is obtained. The second chromatogram (Fig. 6b) shows the product formation when the titration is performed in a medium that promotes bromination

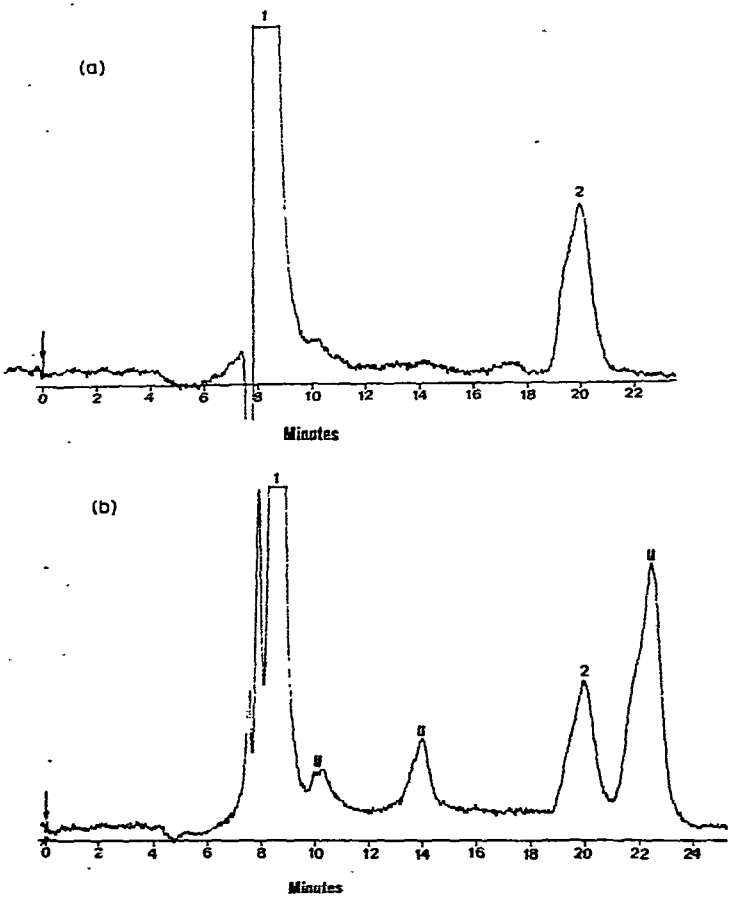



*Fig. 6.* Chromatograms of the product mixtures after bromination of 2,3,5,6-tetramethylphenol in different media. Column: μBondapak $C_{18}$. Eluent: ethanol–water (60:40, v/v). Flow-rate: 20 ml·$h^{-1}$. Wavelength: 280 nm. Volume injected: 5 μl. (a) Titration in the recommended medium[1]. (b) Titration in a more bromination-promoting medium. Peaks: 1 = acetic acid; 2 = 4-bromo-2,3,5,6-tetramethylphenol; U = unknown.

more strongly. Four peaks, including that of the unreacted phenol, can be seen. It is suggested that the two early peaks are due to *p*-quinoid and *o*-quinoid bromocyclohexadienones, while the nature of the compound responsible for the last peak is as yet unknown.

The advantage of the coulometric bromination method is the strict control of the conditions used, especially of the composition of the titration medium and of the rate of bromine generation. The latter circumstance indicates that a local excess of bromine should be avoided. In volumetric bromination methods, these two parameters are not easily controlled and, in fact, the addition of excess of bromine with back-titration of the excess is often recommended. This procedure leads to an increased risk of side-reactions occurring and explains the difficulty of unifying volumetric bromination methods.

*Retention behaviour of brominated alkylphenols*

*Reversed-phase chromatography.* The retention of solutes in reversed-phase chromatography is determined primarily by dispersion forces between the solute and the stationary phase[4,10,11] and by the solubility in the mobile phase[12]. The size and shape of the molecule is of importance for the magnitude of the dispersion forces. Thus, it has been shown that the retention of alkylphenols in reversed-phase chromatography is primarily determined by the size and number of alkyl substituents[4]. Long-chain alkyl groups cause longer retention times than branched-chain groups with the same carbon number, unless these are situated in the *ortho*-position where steric hindrance of the phenolic hydroxyl group can influence the retention.

The introduction of bromine into an alkylphenol gives an increase in the retention in reversed-phase chromatography (see Table II and Figs. 7a, 8a and 9a). This result is in agreement with those in reversed-phase thin-layer chromatography[13,14]. The molar volume of the bromine atom is considered to be of great importance to the retention[13]. It is of interest to note the reversed elution order for 2- and 4-bromophenol in comparison with the corresponding alkylphenols. Thus 2-bromophenol is eluted before 4-bromophenol (Fig. 4), while 4-alkylphenols are eluted earlier than the corresponding *ortho*-isomers[4].

*(a) 2,6-Dialkyl-substituted phenols.* For the 2,6-dialkyl-substituted phenols investigated there is a linear relationship between the capacity factor ($k'$) of the bromination product (4-bromo-2,6-dialkylphenol) and that of the alkylphenol itself, as shown by Fig. 10b. This result can be used, for instance, in the prediction of retention values of unknown bromophenols and for the identification of these products in bromination reactions. The separation factor, $\alpha$, for the bromination product and the alkylphenol decreases linearly with increasing alkyl carbon number ($C_n$), except for 2,6-dimethylphenol (Fig. 11).

*(b) 2,4-Dialkyl-substituted phenols.* On bromination of 2,4-dialkylphenols, bromine enters the free *ortho*-position with the formation of 6-bromo-2,4-dialkylphenols, causing a great change in steric hindrance to the phenolic hydroxyl. However, this change is not reflected in the retention in reversed-phase chromatography, as the linear relationship between the k′ values of brominated and non-brominated 2,6-dialkylphenols is also valid for the 2,4-dialkylphenols investigated, with the exception of 2-*tert.*-butyl-4-methylphenol (Fig. 10b). This fact demonstrates the non-selectivity of this chromatographic system for *ortho*- and *para*-substituted bromophenols.

TABLE II

CAPACITY FACTORS, $k'$, AND SEPARATION FACTORS, $\alpha$, FOR 2,6-, 2,4- AND 4-SUBSTITUTED ALKYLPHENOLS AND THEIR CORRESPONDING BROMINATION PRODUCTS IN THE REVERSED-PHASE LC SYSTEM

Column: μBondapak $C_{18}$. Eluent: ethanol–water (60:40, v/v).

| *Phenol* | $k'$ | | | $\alpha$ | | |
|---|---|---|---|---|---|---|
| | *Non-brominated* | *Monobrominated* | *Dibrominated* | *Mono-/non-* | *Di-/non-* | *Di-/mono-* |
| *2,6-Substituted* | | | | | | |
| 2,6-Dimethyl | 0.6 | 1.07 | | 1.78 | | |
| 2,3,5,6-Tetramethyl | 1.01 | 1.75 | | 1.74 | | |
| 2-Methyl-6-propyl | 1.12 | 1.92 | | 1.71 | | |
| 2-Methyl-6-*tert.*-butyl | 1.52 | 2.51 | | 1.65 | | |
| 2,6-Diisopropyl | 1.71 | 2.67 | | 1.56 | | |
| 2,6-Di-*sec.*-butyl | 2.87 | 4.09 | | 1.43 | | |
| 2,6-Di-*tert.*-butyl | 4.08 | 5.70 | | 1.40 | | |
| *2,4-Substituted* | | | | | | |
| 2,4-Dimethyl | 0.61 | 1.02 | | 1.67 | | |
| 2,4,5-Trimethyl | 0.78 | 1.43 | | 1.83 | | |
| 2-*tert.*-Butyl-4-methyl | 1.52 | 2.94 | | 1.93 | | |
| 2-Methyl-4-*tert.*-hexyl | 2.08 | 3.15 | | 1.51 | | |
| *4-Substituted* | | | | | | |
| Methyl | 0.44 | 0.74 | 1.05 | 1.68 | 2.39 | 1.42 |
| Ethyl | 0.60 | 0.89 | 1.38 | 1.48 | 2.30 | 1.55 |
| Propyl | 0.82 | 1.21 | 1.92 | 1.48 | 2.34 | 1.59 |
| *tert.*-Butyl | 0.92 | 1.30 | 2.02 | 1.41 | 2.20 | 1.55 |
| *sec.*-Butyl | 1.00 | 1.42 | 2.20 | 1.42 | 2.20 | 1.55 |
| *tert.*-Pentyl | 1.23 | 1.68 | 2.55 | 1.37 | 2.07 | 1.52 |
| Cyclohexyl | 1.47 | 2.27 | 3.78 | 1.54 | 2.57 | 1.67 |
| Octyl | 2.53 | 3.27 | 4.83 | 1.29 | 1.91 | 1.48 |

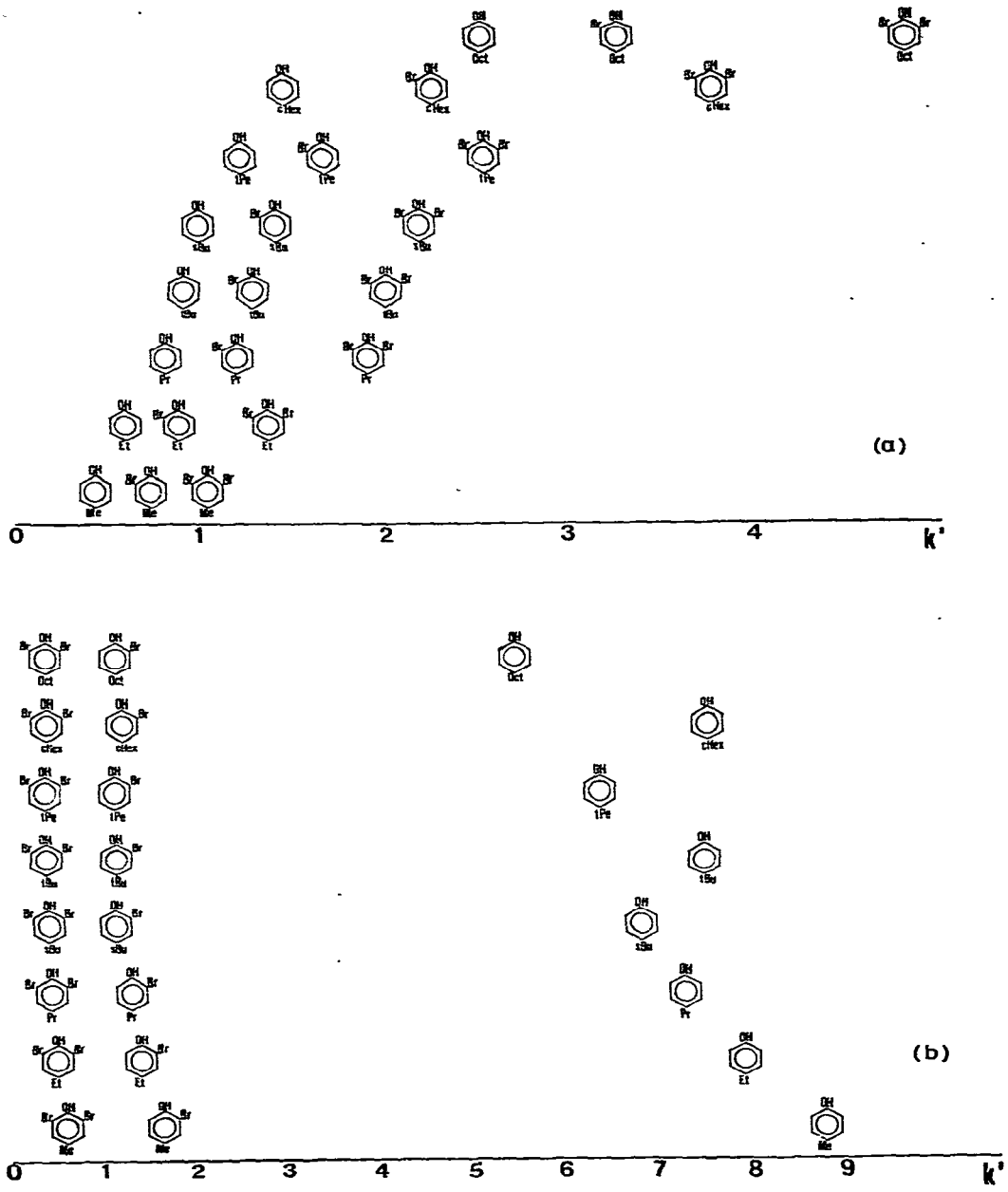



Fig. 7. Retention of 4-alkylphenols and the corresponding bromination products. (a) Reversed-phase LC. Column: $\mu$Bondapak $C_{18}$. Eluent: ethanol–water (60:40, v/v). Flow-rate: 20 ml·h$^{-1}$. (b) Straight-phase LC. Column: Cyano Sil-X-I. Eluent: 0.5% (v/v) 2-propanol in isooctane. Flow-rate: 40 ml·h$^{-1}$.

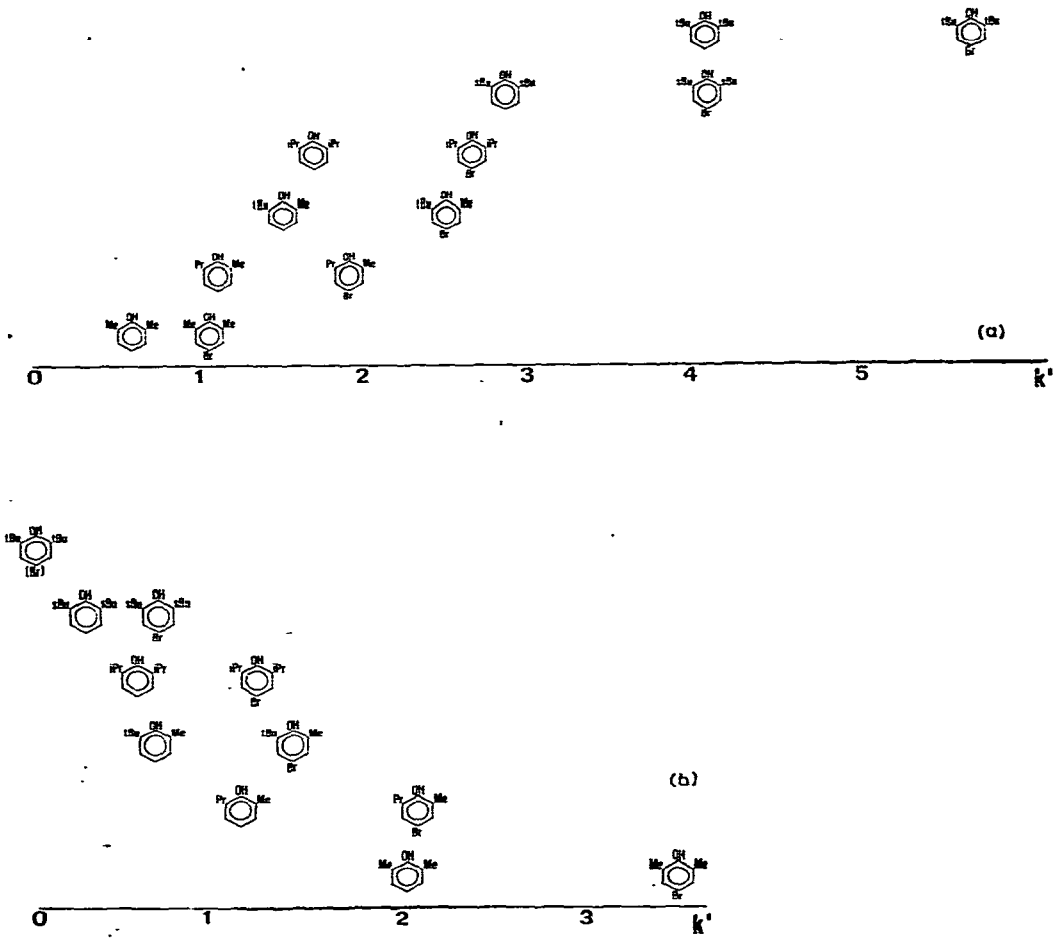



Fig. 8. Retention of 2,6-dialkyl-substituted phenols and the corresponding bromination products. (a) Reversed-phase LC. Column: μBondapak $C_{18}$. Eluent: ethanol–water (60:40, v/v). Flow-rate: 20 ml·h$^{-1}$. (b) Straight-phase LC. Column: Cyano Sil-X-I. Eluent: 0.5% (v/v) 2-propanol in iso-octane. Flow-rate: 40 ml·h$^{-1}$.

*(c) 4-Alkylphenols.* The bromination of 4-alkylphenols yields 2-bromo-4-alkylphenols and 2,6-dibromo-4-alkylphenols. As for the 2,6- and 2,4-dialkylphenols, there is a linear relationship between the $k'$ values of brominated and non-brominated 4-alkylphenols (Fig. 10a). As can be seen, the points for 4-cyclohexylphenol do not lie on the lines, probably owing to the more rigid nature of the cyclic substituent in comparison with the alkyl substituents, which makes the interaction with the reversed-phase LC system different.

The separation factor between mono- and non-brominated phenol is lower for the 4-alkylphenols than for the 2,6-dialkylphenols with the same alkyl carbon number (Fig. 11). The introduction of the second bromine atom results in increasing selectivity between the di- and monobrominated products with increasing alkyl carbon number from $C_1$ to $C_3$, whereafter there is a slight decrease in $\alpha$.

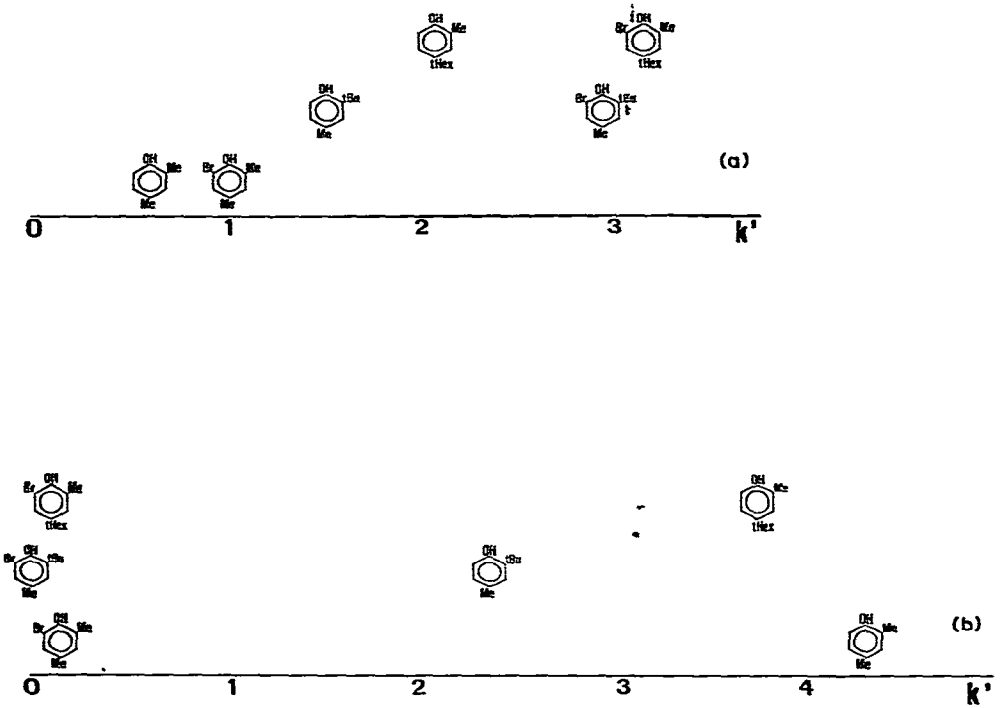



Fig. 9. Retention of 2,4-dialkyl-substituted phenols and the corresponding bromination products. (a) Reversed-phase LC. Column: μBondapak $C_{18}$. Eluent: ethanol–water (60:40, v/v). Flow-rate: 20 ml·h$^{-1}$. (b) Straight-phase LC. Column: Cyano Sil-X-I. Eluent: 0.5% (v/v) 2-propanol in isooctane. Flow-rate: 40 ml·h$^{-1}$.

*Straight-phase liquid chromatography.* It is well known that substituents in the *ortho*-positions have a significant influence on the retention of alkylphenols on polar stationary phases such as silica[15] and nitriles[4]. In fact, on nitrile phases alkylphenols are eluted in the order di-*ortho*-, mono-*ortho*-, non-*ortho*-alkyl-substituted phenols[4]. The introduction of bromine into *ortho*-positions in phenol and alkylphenols results in a drastic decrease in retention, as is illustrated in Figs. 7b and 9b. This effect is due primarily to steric hindrance, but internal hydrogen bonding can also contribute[16]. The introduction of bromine into the *para*-position of an alkylphenol generally leads to an increase in retention[17] (Fig. 8b). Thus, it would be possible to distinguish between different types of alkylphenols by chromatographing them in the straight-phase LC system before and after bromination. In Table III retention data are summarized for alkylphenols and their bromination products on the straight-phase LC system, *i.e.*, on a chemically bonded nitrile phase (Cyano Sil-X-I) with 0.5% (v/v) 2-propanol in isooctane as the mobile phase.

*(a) 2,6-Dialkyl-substituted phenols.* For 2,6-dialkyl-substituted phenols the retention is strongly dependent on the size of the *ortho*-substituents[4]. On the introduction of bromine into the *para*-position the retention increases and, for the phenols investigated, there is a linear relationship between the $k'$ values of the brominated and non-brominated phenols (Fig. 12). The increase in retention on the introduction of bromine is more likely to be due to the increased strength of the hydrogen bond be-

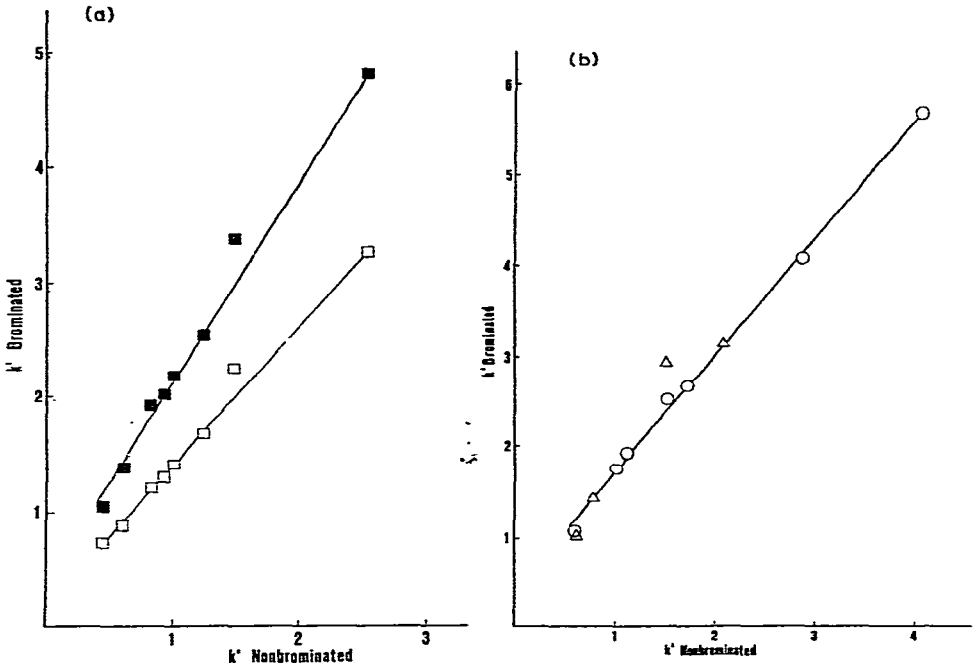



Fig. 10. Relationship between capacity factor, $k'$, for alkylphenols and the corresponding bromination products in reversed-phase LC. Column: μBondapak $C_{18}$. Eluent: ethanol–water (60:40, v/v). Flow-rate: 20 ml·h$^{-1}$. (a) $k'$ for brominated 4-alkylphenol *vs.* $k'$ for the alkylphenol itself. □, $k'$ for monobrominated *vs.* $k'$ for non-brominated; ■, $k'$ for dibrominated *vs.* $k'$ for non-brominated. (b) $k'$ for brominated 2,6- and 2,4-alkylphenols *vs.* $k'$ for the alkylphenol itself. ○, 2,6-Dialkyl-substituted phenols; △, 2,4-dialkyl-substituted phenols.

tween the phenolic hydroxyl group and the stationary phase than to interactions between the bromine atom and the stationary phase[17]. This is indicated by the fact that for 2,6-di-*tert.*-butylphenol, in which the hydroxyl group is completely sterically hindered, the introduction of bromine has no effect on retention.

The separation factor for a 2,6-dialkylphenol and its corresponding brominated product increases slightly with increasing size of the substituent, *i.e.*, with the alkyl carbon number ($C_n$), which is shown by the lower line in Fig. 13.

*(b) 2,4-Dialkyl-substituted phenols and their bromination products.* The introduction of bromine takes place in the free *ortho*-position, which has a great effect on the $k'$ values, causing a decrease by a factor of 30 or more for the three phenols investigated (see Fig. 9b and values of $\alpha$ in Table III). Steric hindrance by the bromine atom is probably the dominating factor which contributes to decreased retention.

*(c) 4-Alkylphenols and their bromination products.* The introduction of one bromine atom results in a significant decrease in retention (see Fig. 7b and Table III), *i.e.*, a separation factor of 5–6 between the non- and monobrominated product is obtained while the introduction of a second bromine atom has a lesser effect, giving $\alpha \approx 3$ between di- and monobrominated alkylphenols. This effect can be compared with the changes in retention caused by introducing bromine into the *ortho*-position in 2,4-dialkyl-substituted phenols, where the effect is much greater ($\alpha \approx 30$–50). As

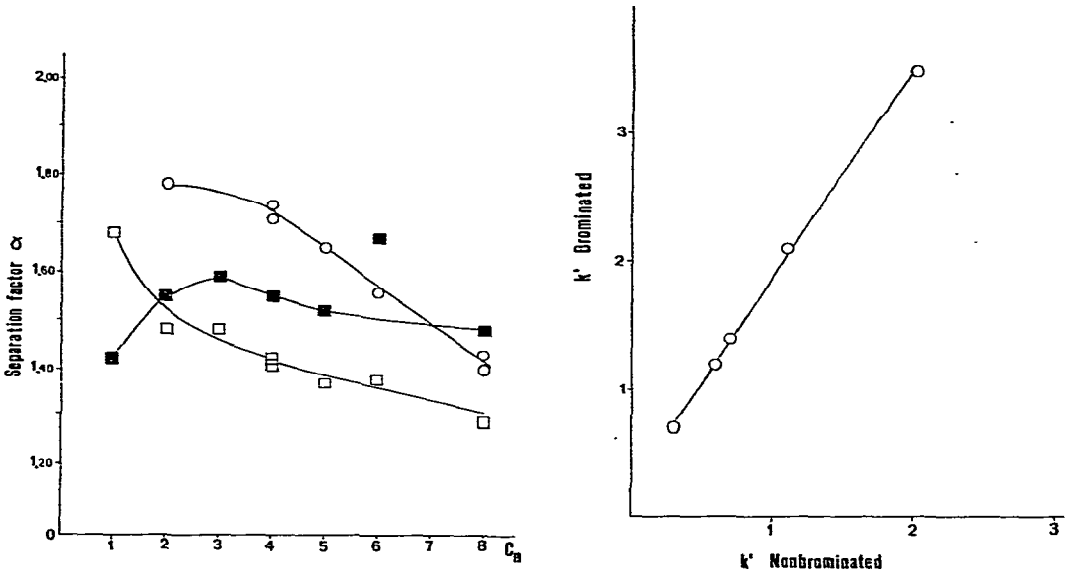



Fig. 11. Relationship between the separation factor, $\alpha$, for brominated and non-brominated and for dibrominated and monobrominated phenols, and alkyl carbon number. Column: $\mu$Bondapak $C_{18}$. Eluent: ethanol–water (60:40, v/v). Flow-rate: 20 ml·h$^{-1}$. ○, 2,6-Dialkyl-substituted phenols; □, mono-/non-brominated 4-alkylphenol; ■, di-/monobrominated 4-alkylphenol.

Fig. 12. Capacity factor, $k'$, for brominated 2,6-dialkylphenol *vs.* $k'$ for the alkylphenol itself. Column: Cyano Sil-X-I. Eluent: 0.5% (v/v) 2-propanol in isooctane. Flow-rate: 40 ml·h$^{-1}$.

can be expected, there is no significant correlation between the size of the *para*-substituent and the separation factor for non- and monobrominated and mono- and dibrominated 4-alkylphenol, respectively, as illustrated in Fig. 13.

*Resolution of mixtures of 3- and 4-alkylphenols after bromination.* Mixtures of 3- and 4-alkylphenols are often difficult to separate as such by LC. However, by converting the phenols into their fully brominated derivatives, a mixture of bromophenols is obtained that can easily be separated by LC, as demonstrated for 3- and 4-methylphenol in Fig. 14. The phenolic mixture was coulometrically brominated using the full bromination conditions given under Experimental and 100 $\mu$l of the reaction mixture were then injected directly on to the $\mu$Bondapak $C_{18}$ column. The bromination resolution method is also applicable to mixtures of other alkylphenols provided that they can be coulometrically monobrominated or fully brominated using the same conditions, and both quantitative and qualitative analyses are possible.

## CONCLUSIONS

For phenol, 4-alkylphenols and 2,4- and 2,6-dialkyl-substituted phenols the coulometric bromination technique described by Kinberger *et al.*[1] yields predictable unambigous products when the titration is continued to the end-point. In the reaction, hydrogen is exchanged for bromine at free *ortho*- and *para*-positions, with the formation of *o*- and *p*-bromophenols.

TABLE III

CAPACITY FACTORS, $k'$, AND SEPARATION FACTORS, $\alpha$, FOR 2,6-, 2,4- AND 4-SUBSTITUTED ALKYLPHENOLS AND THEIR CORRESPONDING BROMINATION PRODUCTS IN THE STRAIGHT-PHASE LC SYSTEM

Column: Cyano Sil-X-I. Eluent: 0.5% (v/v) 2-propanol in isooctane.

| *Phenol* | $k'$ | | | $\alpha$ | | | |
|---|---|---|---|---|---|---|---|
| | *Non-brominated* | *Monobrominated* | *Dibrominated* | *Mono-/non-* | *Non-/mono-* | *Non-/di-* | *Mono-/di-* |
| *2,6-Substituted* | | | | | | | |
| 2,6-Dimethyl | 2.0 | 3.5 | | 1.7 | | | |
| 2-Methyl-6-propyl | 1.1 | 2.1 | | 1.9 | | | |
| 2-Methyl-6-*tert.*-butyl | 0.7 | 1.4 | | 2.2 | | | |
| 2,6-Diisopropyl | 0.6 | 1.2 | | 2.2 | | | |
| 2,6-Di-*sec.*-butyl | 0.3 | 0.7 | | 2.5 | | | |
| 2,6-Di-*tert.*-butyl | —* | —* | | — | | | |
| *2,4-Substituted* | | | | | | | |
| 2,4-Dimethyl | 4.3 | 0.15 | | | 31 | | |
| 2-*tert.*-Butyl-4-methyl | 2.4 | ~0.05 | | | ~50 | | |
| 2-Methyl-4-*tert.*-hexyl | 3.7 | 0.10 | | | 37 | | |
| *4-Substituted* | | | | | | | |
| Methyl | 8.8 | 1.6 | 0.6 | | 5.4 | 14.9 | 2.8 |
| Ethyl | 7.9 | 1.4 | 0.5 | | 5.7 | 16.5 | 2.9 |
| Propyl | 7.3 | 1.2 | 0.4 | | 5.6 | 17.3 | 3.1 |
| *tert.*-Butyl | 7.5 | 1.1 | 0.35 | | 6.7 | 20.8 | 3.1 |
| *sec.*-Butyl | 6.8 | 1.1 | 0.4 | | 6.1 | 17.4 | 2.9 |
| *tert.*-Pentyl | 6.4 | 1.1 | 0.35 | | 5.7 | 18.2 | 3.2 |
| Cyclohexyl | 7.5 | 1.2 | 0.4 | | 6.3 | 19.8 | 3.2 |
| Octyl | 5.5 | 1.1 | 0.35 | | 4.8 | 15.1 | 3.1 |

* Unretained.

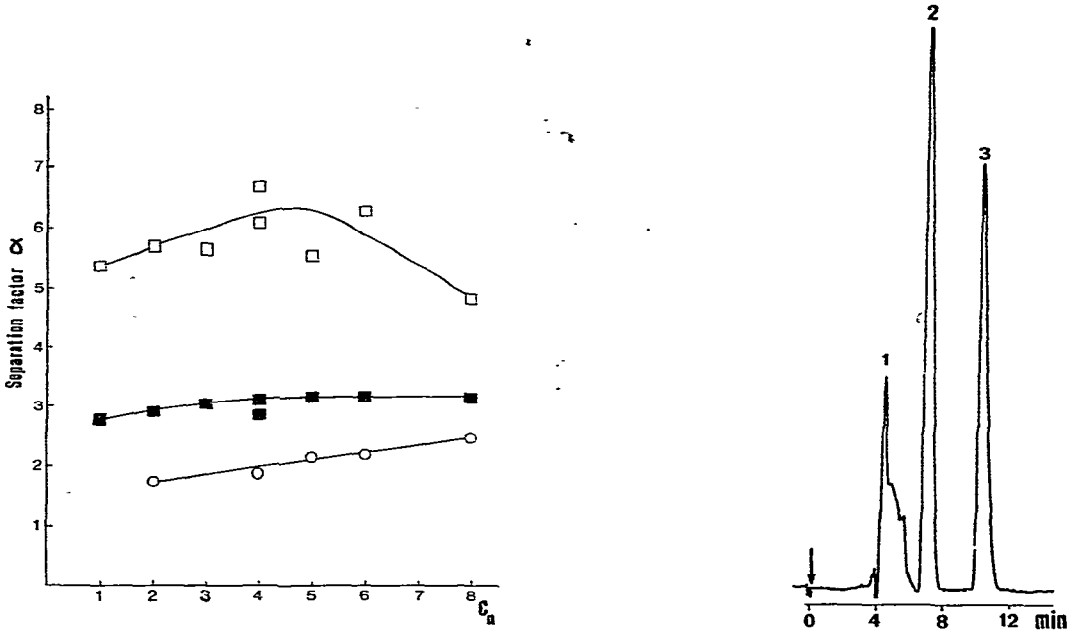



Fig. 13. Relationship between the separation factor, $\alpha$, for brominated and non-brominated and for monobrominated and dibrominated phenols, and alkyl carbon number. Column: Cyano Sil-X-I. Eluent: 0.5% (v/v) 2-propanol in isooctane. Flow-rate: 40 ml·h$^{-1}$. ○, Mono-/non-brominated 2,6-dialkylphenols; □, non-/monobrominated 4-alkylphenols; ■, mono-/dibrominated 4-alkylphenols.

Fig. 14. Chromatogram of the product mixture after full bromination of a mixture of 3- and 4-methylphenol. Column: $\mu$Bondapak $C_{18}$. Eluent: ethanol–water (70:30, v/v). Flow-rate: 40 ml·h$^{-1}$. Volume injected: 100 $\mu$l. Peaks: 1 = acetic acid; 2 = 2,6-dibromo-4-methylphenol; 3 = 2,4,6-tribromo-3-methylphenol.

Over-bromination leads to further nuclear bromination in still free *ortho*- and *para*-positions or to exchange of alkyl groups, *e.g.*, *tert.*-butyl groups, in the same positions for bromine. In another reaction mode, quinoid bromocyclohexadienones are formed on over-bromination. 2,6-Dimethyl-substituted phenols seem to be especially prone to give this reaction.

It is important to use the correct titration medium, as a medium that promotes bromination too strongly leads to side-reactions with the formation of non-regular bromination products, *e.g.*, bromocyclohexadienones.

The reversed-phase microparticulate LC system is the most suitable for combination with coulometric bromination of phenols, in that the sample can be injected directly on to the column without any noticeable effect on column performance. Introduction of bromine into the *ortho*- or *para*-positions of an alkylphenol causes an increase in retention in the reversed-phase LC system. There is a linear relationship between reversed-phase $k'$ values of brominated and non-brominated phenols which is of value for the identification of alkylphenols after bromination.

Introduction of bromine into the *para*-position of an alkylphenol increases the retention in the straight-phase LC system, while substitution of bromine in the *ortho*-position causes a marked decrease in retention. This fact is of diagnostic value in alkylphenol research.

Coulometric bromination can be used for the transformation of mixtures of alkylphenols, which are difficult to separate as such by LC, into easily resolved mixtures of bromophenols.

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