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LIQUID CHROMATOGRAPHY STUDY OF BROMINATED ANILINES AND INVESTIGATION OF PRODUCT FORMATION IN THE BROMINATION REACTION

I. ANILINES WITHOUT RING SUBSTITUENTS OR WITH ALKYL GROUPS IN THE *ortho*- AND *para*-POSITIONS

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SUMMARY

Straight- and reversed-phase liquid chromatography (LC) have been used to study product formation in the quantitative coulometric bromination of various anilines. The coulometric method yields, up to the end-point, predictable unambiguous products by exchange of hydrogen for bromine in free *ortho*- and *para*-positions. After the end-point, oxidation products may be formed from primary anilines and N-alkyl groups splitt off from secondary and tertiary anilines.

A detailed study of retention behaviour of some 70 bromoanilines in straight- and reversed-phase LC has been made. An increase in retention generally took place on the introduction of bromine into the aniline nucleus, except for the formation of *o*-bromoanilines in straight-phase LC, where retention decreases. Retention behaviour of bromoanilines in straight-phase LC is discussed in terms of base strength, steric hindrance around the nitrogen atom and solvent effects. A semi-linear relationship was found to exist between capacity factors of brominated and non-brominated anilines in both the LC systems.

INTRODUCTION

In a previous paper[1] it was shown that alkylanilines can be quantitatively brominated by a coulometric technique, based on reaction with anodically generated bromine. The advantage of the 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 anilines, even those that are usually sensitive to side-reactions such as oxidation.

The aims of this investigation were to study, by liquid chromatography (LC), product formation at various stages during the coulometric bromination, and to

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examine the retention behaviour of brominated alkylanilines in different LC systems. The choice of anilines was restricted to compounds that either have no ring substituents or contain alkyl groups in the *ortho*- and/or *para*-positions, *i.e.* 2- and 4-monoalkylanilines and 2,4- and 2,6-dialkylanilines. Primary, secondary and tertiary anilines are included. In Part II[3] *meta*-alkyl-substituted alkylanilines are considered.

EXPERIMENTAL

Apparatus

Coulometric titration. The apparatus and procedure for the coulometric bromination have been described in detail elsewhere[1,4].

Liquid chromatography. The LC pump used was a Varian Model 4100 (Varian, Palo Alto, CA, U.S.A.) and the detector was a Laboratory Data Control Model 1285 UV monitor (Laboratory Data Control, Riviera Beach, FL, U.S.A.) used at 280 nm. Sample application was accomplished by a valve injector (Rheodyne, Berkeley, CA, U.S.A.) with a 20-μl loop.

Columns. The bonded-phase packing materials were the commercially available Nucleosil C_{18} (5 μm) and Nucleosil CN (5 μm) (Macherey, Nagel & Co., Düren, G.F.R.), packed by the upward-slurry packing technique[5,6]. All columns consisted of precision-bore stainless-steel tubing (200 mm × 4.4 mm I.D.) and were used at room temperature.

For accurate work it is necessary to reactivate the columns, especially the nitrile column, regularly. This was made according to recommended procedures. The stability of the columns was tested daily using a mixture of phenol, 2,6-dimethylphenol and 4-*tert.* butylphenol for the reversed-phases and a mixture of diphenylamine, triphenylamine and N-methyl-2-methylaniline for the nitrile phase.

Chemicals

Isooctane (certified ACS grade, Fisher Scientific, Fairlawn, NJ, U.S.A.), methanol (analytical-reagent grade, May & Baker, Dagenham, Great Britain), 2-propanol (*pro analysi* grade, E. Merck, Darmstadt, G.F.R.), sodium dihydrogen phosphate, $NaH_2PO_4,2H_2O$ (99%, BDH, Poole, Great Britain), disodium hydrogen phosphate, $Na_2HPO_4,2H_2O$ (according to Sörensen, E. Merck) and orthophosphoric acid (*pro analysi* grade, E. Merck) were used for preparing the LC eluents.

Acetic acid (*pro analysi* grade, E. Merck) and sodium bromide (99%, BDH) were used for the preparation of the titration media.

The anilines were of the best grade commercially available. Some of them were further purified by distillation or recrystallization. N,N-Dimethyl-2,6-dimethylaniline, N,N-diethyl-2-ethylaniline and N-ethyl-2-ethylaniline were prepared at this laboratory.

Mobile phases

Methanol–aqueous buffer was used for reversed-phase LC on the C_{18} phase, and isooctane, containing 0.2% (v/v) 2-propanol, was applied for straight-phase LC on the nitrile phase.

Methanol–aqueous buffer (70:30, v/v, pH 7.0). This buffer was made from 15 ml of 0.025 *M* Na_2HPO_4, 250 ml of 0.025 *M* NaH_2PO_4, and 618.33 ml of methanol;

the pH was adjusted to 7.0 with small amounts of orthophosphoric acid and dilute sodium hydroxide (1 *M*).

Methanol–aqueous buffer (80:20, v/v, pH 7.0). This buffer was made from 15 ml of 0.05 *M* Na_2HPO_4, 250 ml of 0.05 *M* NaH_2PO_4 and 1060 ml of methanol; the pH was adjusted as above.

Procedure

Coulometric titration. Anilines, *ca.* 20 μequiv. in 20 ml of titration medium, were coulometrically brominated as described by Truedsson and Smith[1]. All titrations were performed in medium III-1 (acetic acid–water, 60:40, v/v), bromide concentration 0.1 *M*.

Chromatography. For the reversed-phase studies, 20-μl aliquots containing *ca.* 5 nmol 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 samples were then injected directly on to the column. In the case of the nitrile phase studies, an extraction procedure was included in order to remove the acetic acid and water.

Extraction procedure. An aliquot (4 ml) of the titration mixture was transferred to a 50-ml separating funnel, and 5 ml of water and 5 ml of isooctane were added. After shaking, sodium hydroxide (10 *M*, 4–5 ml) was added in order to make the water phase alkaline, and the mixture was shaken again. The isooctane phase was removed and dried over sodium sulphate, and the solvent was partly evaporated with a gentle flow of nitrogen to a volume of 1–2 ml. From this solution, 20 μl were injected on to the nitrile phase column.

Capacity factor, k′. The capacity factor given is a mean value from at least three injections with a relative standard deviation of *ca.* 3%. Retention times of unretained solutes were determined by injecting *n*-hexane on the nitrile phase and aqueous sodium nitrate solution (0.05%) on the reversed phases. The capacity factor k' was calculated from the following formula

$$k' = \frac{t_R - t_0}{t_0} \tag{1}$$

where t_R is the retention time of sample and t_0 the retention time of unretained solute.

Separation factor, α. The separation factor for a pair of substances A and B is given by ratio of their capacity factors

$$\alpha_{A,B} = \frac{k'_A}{k'_B} \tag{2}$$

RESULTS AND DISCUSSION

Choice of liquid chromatographic systems

In a previous investigation[7] it was shown that valuable information about the structure of different kinds of aniline can be gained by a combination of straight- and reversed-phase LC.

For the study of brominated anilines in this work the same kinds of chromato-

graphic systems were chosen, *viz.* one straight-phase system on nitrile phase with isooctane, containing 0.2% (v/v) 2-propanol, as eluent and one reversed-phase octadecylsilane (C_{18}) system with methanol–aqueous buffer as eluent. Two eluents were used, one with methanol–aqueous buffer (70:30, v/v) and one with the proportions 80:20 (v/v). In both cases the eluents were buffered at pH 7.0 in order to improve peak symmetry and reduce the tendency to formation of double peaks.

It appeared, on varying the methanol content in the eluent, that the retention of brominated anilines on the C_{18} phase decreased when the percentage of methanol increased. In order to obtain appropriate elution times and good resolution, the composition methanol–aqueous buffer (70:30, v/v) was chosen for brominated primary anilines and the composition 80:20 (v/v) for brominated secondary and tertiary anilines. However, for comparison some primary anilines were also run with the latter eluent. On the nitrile phase, retention of brominated anilines is very much dependent on the content of 2-propanol in the isooctane eluent. On that account, the eluent must be carefully prepared and it is also essential to use dry solvents.

Introduction of sample. On the reversed-phase column, direct injection of aliquots taken from the titration vessel was carried out without any disturbances of the chromatogram or impairment in column performance. On the straight-phase system, however, an extraction procedure had to be carried out before injection, in order to remove acetic acid and water as previously described.

Product formation in coulometric bromination

In the coulometric bromination method for the titration of anilines described by the present authors[1], the reaction is carried out in a water–acetic acid medium and the reactivity is controlled by varying the water content and the bromide ion concentration and by the addition of pyridine. The reaction is believed to involve substitution with bromine at free *ortho*- and *para*-positions. For anilines with several such positions, the titration can be carried out either to the fully brominated stage or to a stage corresponding to the introduction of a smaller number of bromine atoms than the number of available free *ortho*- and *para*-positions. For a certain aniline, the outcome of a titration is dependent both on the structure and on the bromination-promoting properties of the titration medium.

In the present investigation, the aim was to study product formation during

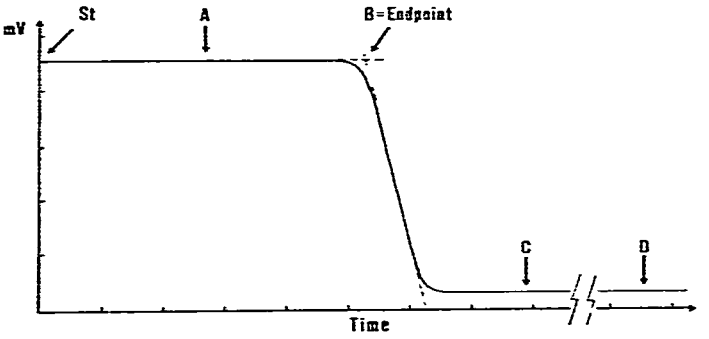

Fig. 1. Titration curve for coulometric bromination of alkylanilines. St, A, B, C and D indicate points at which samples were removed from the titration vessel.

titration in the pyridine-free medium III-1, containing acetic acid and water in the proportions 60:40 (v/v) and with a bromide concentration of 0.1 *M*. For this purpose samples were removed from the titration vessel at various stages of the titration and injected on to the LC columns either directly (C_{18} phase) or after extraction (nitrile phase). Fig. 1, which gives a typical titration curve, illustrates the procedure. Samples were removed at points indicated by A, B, C and D, and analyzed. The resulting chromatograms obtained for 2-methylaniline can be seen in Fig. 2. At point A, half-way to the end-point, the main bromination products formed are 4-bromo-2-methylaniline (c) and 4,6-dibromo-2-methylaniline (e), while very little of the 6-bromo derivative (d) appears. At the end-point (B) only the dibrominated product (e) is present. Analogous results were obtained for other 2-alkylanilines when titrated in medium III-1.

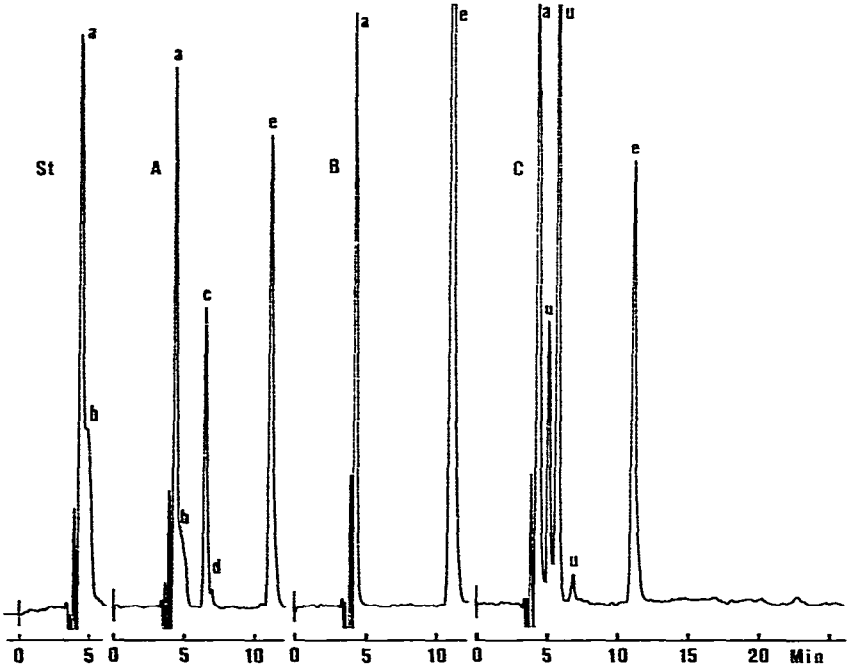

Fig. 2. Chromatograms of the product mixture after bromination of 2-methylaniline. Column, Nucleosil C_{18}; eluent, methanol–aqueous buffer (80:20, v/v, pH 7.0), 45 ml h^{-1}; wavelength, 280 nm; volume injected, 20 μl. Peaks: a = acetic acid; b = 2-methylaniline; c = 4-bromo-2-methylaniline; d = 6-bromo-2-methylaniline; e = 4,6-dibromo-2-methylaniline; u = unknown compound. St, A, B and C refer to Fig. 1.

The results of the monobromination of different kinds of aniline up to point A on the titration curve can be gathered from Table I, which gives the k' values on the C_{18} phase of the various bromoanilines formed. As can be seen, all tested primary anilines with a free *ortho*-position yield an *ortho*-bromo derivative, while secondary and tertiary anilines furnish this derivative only when the *para*-position is occupied by an alkyl group. The increased steric hindrance at the *ortho*-positions of secondary and tertiary anilines obviously precludes the introduction of a bromine atom, unless the *para*-position is occupied.

Diphenylamine constitutes an exception from other secondary anilines in that

an *ortho*-bromo derivative is formed on bromination in medium III-1. Samples taken during the bromination show that the order of formation of bromo derivatives is 2- and 4-monobromo-, 2,4′- and 4,4′-dibromo-, 2,4,4′-tribromo- and 2,2′,4,4′-tetrabromodiphenylamine.

Triphenylamine, however, does not yield any *ortho*-bromo derivative. In this case the order of formation of bromo derivatives is 4-monobromo-, 4,4′-dibromo- and 4,4′,4″-tribromotriphenylamine.

Effect of over-bromination. All evidence points to the fact that coulometric bromination of anilines up to the end-point in the proper titration medium proceeds with the formation of bromoanilines owing to exchange of hydrogen for bromine at free *ortho*- and *para*-positions. However, it is well known that, in the analysis of anilines by volumetric bromination, some compounds are able to consume more bromine than the stoichiometric amount corresponding to the free *ortho*- and *para*-positions. Examples of side-reactions that have been suggested or proved to take place are replacement of *ortho*- and *para*-situated groups with bromine and various oxidation reactions[8–11].

The reason for the good quantitative results obtained on coulometric bromination of anilines, in comparison with results from volumetric bromination, is undoubtedly the careful choice of proper titration media and the fact that an excess of bromine is never allowed to build up before the end-point. Nevertheless, it was considered to be of interest also to examine the behaviour of the anilines in question after the end-point. For this purpose an excess of bromine was generated which depended on the kind of aniline. Thus, for primary anilines, where all vacant *ortho*- and *para*-positions are substituted with bromine at the end-point, an excess of *ca.* 50% of bromine was generated (point C in Fig. 1). Secondary and tertiary anilines, which are not fully brominated in medium III-1, were exposed to an excess of bromine corresponding to full bromination (point D in Fig. 1).

Among primary anilines, *ortho*-alkyl-substituted compounds seem to be particularly prone to react further on over-bromination. Thus, 2,6-dimethylaniline formed an over-bromination product which was investigated by means of gas chromatography–mass spectrometry and nuclear magnetic resonance spectroscopy and shown to be 4-amino-4′-bromo-3,5,2′,6′-tetramethyldiphenylamine (b in Fig. 3). It is accordingly an oxidation product from 4-bromo-2,6-dimethylaniline, which latter compound is present at the end-point. This oxidation product is formed soon after the end-point; in fact, a small amount is present even before. We have also found it to arise on volumetric over-bromination and similar structures have been isolated on anodic oxidation of *p*-chloroaniline[12].

Further generation of bromine in the reaction mixture of 2,6-dimethylaniline yielded another product which, on the nitrile phase, was eluted nearer the front than the diphenylamine above (a in Fig. 3). It was tentatively identified as 4,4′-dibromo-2,6,2′,6′-tetramethylhydrazobenzene, a type of compound which has been reported to be formed on anodic oxidation of anilines[12]. As shown by the small peaks (u in Fig. 3), other over-bromination products are formed, the nature of which, however, is as yet unknown.

On over-bromination of 2-methylaniline, some early peaks of unknown origin appear at separation on the C_{18} phase (u in Fig. 2C). They are formed from 2-methyl-4,6-dibromoaniline (e) and spectral evidence indicates that their structure is quin-

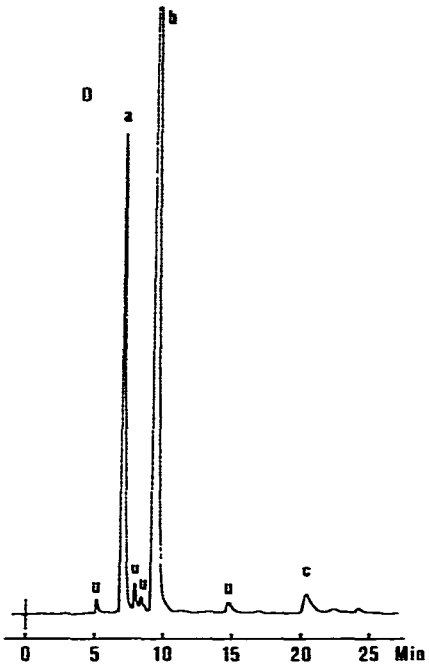

Fig. 3. Chromatogram of the product mixture after over-bromination of 2,6-dimethylaniline. Column, Nucleosil CN; eluent, 0.2% (v/v) 2-propanol in isooctane, 45 ml h^{-1}; wavelength, 280 nm; volume injected, 20 μl. Peaks: a = 4,4'-dibromo-2,6,2',6'-tetramethylhydrazobenzene; b = 4-amino-4'-bromo-3,5,2',6'-tetramethyldiphenylamine; c = 4-bromo-2,6-dimethylaniline; u = unknown compound. D refers to Fig. 1.

oidic. These peaks are late on the nitrile phase in contrast to the substituted diphenylamine and hydrazobenzene, previously discussed in connection with over-bromination of 2,6-dimethylaniline (a and b in Fig. 3).

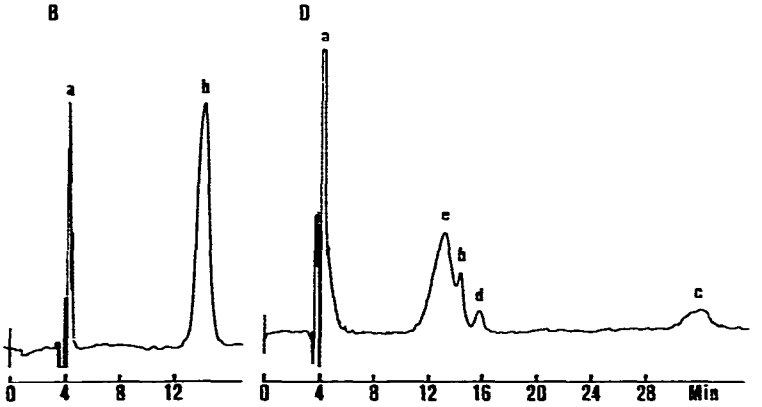

Fig. 4. Chromatogram of the product mixture after bromination of N,N-dimethyl-2-methylaniline. Column, Nucleosil C_{18}; eluent, methanol–aqueous buffer (80:20, v/v, pH 7.0), 45 ml h^{-1}; wavelength, 280 nm; volume injected, 20 μl. Peaks: a = acetic acid; b = N,N-dimethyl-2-methyl-4-bromoaniline; c = N,N-dimethyl-2-methyl-4,6-dibromoaniline; d = N-methyl-2-methyl-4,6-dibromoaniline; e = 2-methyl-4,6-dibromoaniline. B and D refer to Fig. 1.

TABLE I

CAPACITY FACTORS AND SEPARATION FACTORS FOR *ortho*- AND *para*-ALKYL-SUBSTITUTED ANILINES AND THEIR BROMINATION PRODUCTS IN THE REVERSED-PHASE LC SYSTEM

Column, Nucleosil C_{18}; eluent, methanol–aqueous buffer (80:20 and 70:30*, v/v, pH 7.0)

No.	*Aniline (substituent)*	*Capacity factor, k'*						*Separation factor, α*					
		Non-brominated	*Monobrominated*		*Dibrominated*		*Tri-brominated*	*Mono-/non-*		*Di-/mono-ortho-*		*Di-/mono-para*	*Tri-/di-*
			Ortho	*Para*	*Di-ortho*	*Ortho-para*		*Ortho*	*Para*	*Di-ortho*	*Ortho-para*	*Ortho-para*	
1	None*	0.55	1.33	1.16		3.33	8.87	2.42	2.11		2.50	2.87	2.66
1,	None	0.41					3.48						
2	2-Methyl*	0.85	2.20	1.82		5.68		2.59	2.14		2.58	3.12	
2	2-Methyl	0.55	1.10	0.96		2.35		2.00	1.75		2.14	2.45	
3	2-Ethyl*	1.26	3.14	2.54		7.87		2.49	2.02		2.51	3.10	
4	2-Isopropyl*	1.69	4.20	3.33		10.2		2.49	1.97		2.43	3.06	
5	4-Methyl*	0.86	2.01		5.36			2.34		2.67			
6	4-Ethyl*	1.26	3.04		8.25			2.41		2.71			
7	4-Isopropyl*	1.80	4.08		10.8			2.27		2.65			
8	4-*n*-Butyl*	3.29	7.84		21.5			2.38		2.74			
9	2,4-Dimethyl*	1.25	3.36					2.69					
10	2-Methyl-4-butyl*	5.02	12.7					2.53					
11	2,6-Dimethyl*	1.31		2.89					2.21				
11	2,6-Dimethyl	0.78		1.39					1.78				
12	N-Methyl	0.68		1.25		3.04	5.14		1.84			2.43	1.69
13	N-Ethyl	0.87		1.57		4.39	7.18		1.80			2.80	1.64
14	N-Propyl	1.23		2.26		6.46	11.3		1.84			2.86	1.75

15	N-*n*-Butyl	1.69		2.79		8.87	15.3		1.65		3.18	1.72
16	N-Phenyl	1.52	3.20	2.79	4.89**	4.71***	8.75‡	2.11	1.84			
17	N-Benzyl	1.50		2.61		6.55	12.3		1.74		2.51	1.88
18	N-Methyl-2-methyl	0.97		1.83		3.80			1.89		2.08	
19	N-Ethyl-2-methyl	1.34		2.48		5.30			1.85		2.14	
20	N-Ethyl-2-ethyl	1.86		3.23		6.90			1.74		2.14	
21	N-Methyl-4-methyl	0.90	2.17		2.95			2.41		1.36		
22	N-Ethyl-4-methyl	1.15	3.26		4.20			2.83		1.29		
23	N,N-Dimethyl	1.35		2.45		3.64	5.02		1.81		1.49	1.38
24	N,N-Diethyl	2.32		4.02		7.06			1.73		1.76	
25	N,N-Diphenyl	7.40		13.0		21.4***	31.9‡‡		1.76		1.65	1.49
26	N,N-Dimethyl-2-methyl	1.76		3.20		8.91			1.82		2.78	
27	N,N-Diethyl-2-methyl	3.45		6.40		16.2			1.86		2.53	
28	N,N-Diethyl-2-ethyl	5.21		8.94		21.8			1.72		2.44	
29	N,N-Dimethyl-4-methyl	1.80	2.32		7.37			1.29		3.18		
30	N,N-Diethyl-4-methyl	2.95	4.61		13.5			1.56		2.93		
31	N,N-Dimethyl-2,6-dimethyl	4.20		7.84					1.87			

* 70:30 eluent.
** 2,4′-Dibrominated.
*** 4,4′-Dibrominated.
‡ 2,4,4′-Tribrominated; 2,4,2′,4′-tetrabrominated; $k' = 2.03$.
‡‡ 4,4′,4″-Tribrominated.

para-Alkyl-substituted primary anilines show a far better stability against excess of bromine than *ortho*-substituted. The 4-alkylanilines, for example, gave essentially the same chromatogram at 50% over-bromination as at the end-point.

Secondary and tertiary anilines of most of the kinds studied in this work are not fully brominated at the end-point in medium III-1, but still contain vacant *ortho*-positions. The result of over-bromination is that these free positions are substituted with bromine, producing the fully brominated aniline. However, this reaction cannot be utilized for quantitative purposes. The fully brominated secondary and tertiary anilines are very sensitive to further excess of bromine and react with loss of N-alkyl groups to the corresponding primary and secondary anilines (Fig. 4). An exception from this reaction order was constituted by N,N-diethylaniline. This compound is present as the 4-bromo derivative at the end-point and, on additional generation of bromine, one of the N-ethyl groups is split off, before further bromination takes place.

Retention behaviour of brominated anilines

Reversed-phase chromatography. The retention of solutes in reversed-phase chromatography is determined primarily by dispersion forces between the solute and the stationary phase and by the solubility in the mobile phase[13–15]. The size and shape

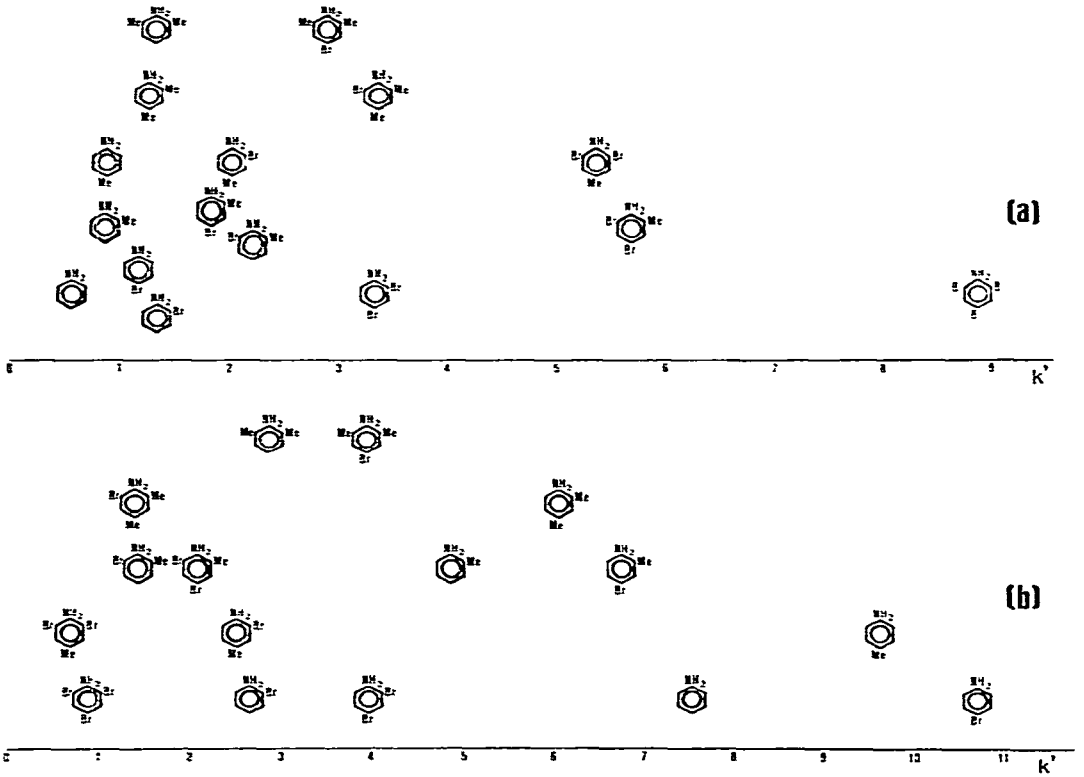

Fig. 5. Retention of primary anilines and the corresponding bromination products. (a) Reversed-phase LC. Column, Nucleosil C_{18}; eluent, methanol–aqueous buffer (80:20, v/v, pH 7.0), 45 ml h^{-1}. (b) Straight-phase LC. Column, Nucleosil CN; eluent, 0.2% (v/v) 2-propanol in isooctane; 45 ml h^{-1}. Me = Methyl.

of the molecule is of importance for the magnitude of the dispersion forces. Thus, it has been shown that the retention of primary, secondary and tertiary anilines in reversed-phase chromatography is mainly determined by the total alkyl carbon number, representing the sum of the nuclear and N-substituted alkyl groups[7].

The introduction of bromine into the aniline nucleus gives a considerable increase in retention in reversed-phase chromatography (Table I and Figs. 5a and 6a). The great molar volume of the bromine atom is considered to be of special importance for this change in retention, decreasing the solubility of the bromoanilines in the eluent[16].

There exists a semi-linear relationship between k' values of the original anilines on one hand, and k' values of the corresponding mono-, di- and tribrominated derivatives, respectively, on the other hand, as demonstrated by Fig. 7.

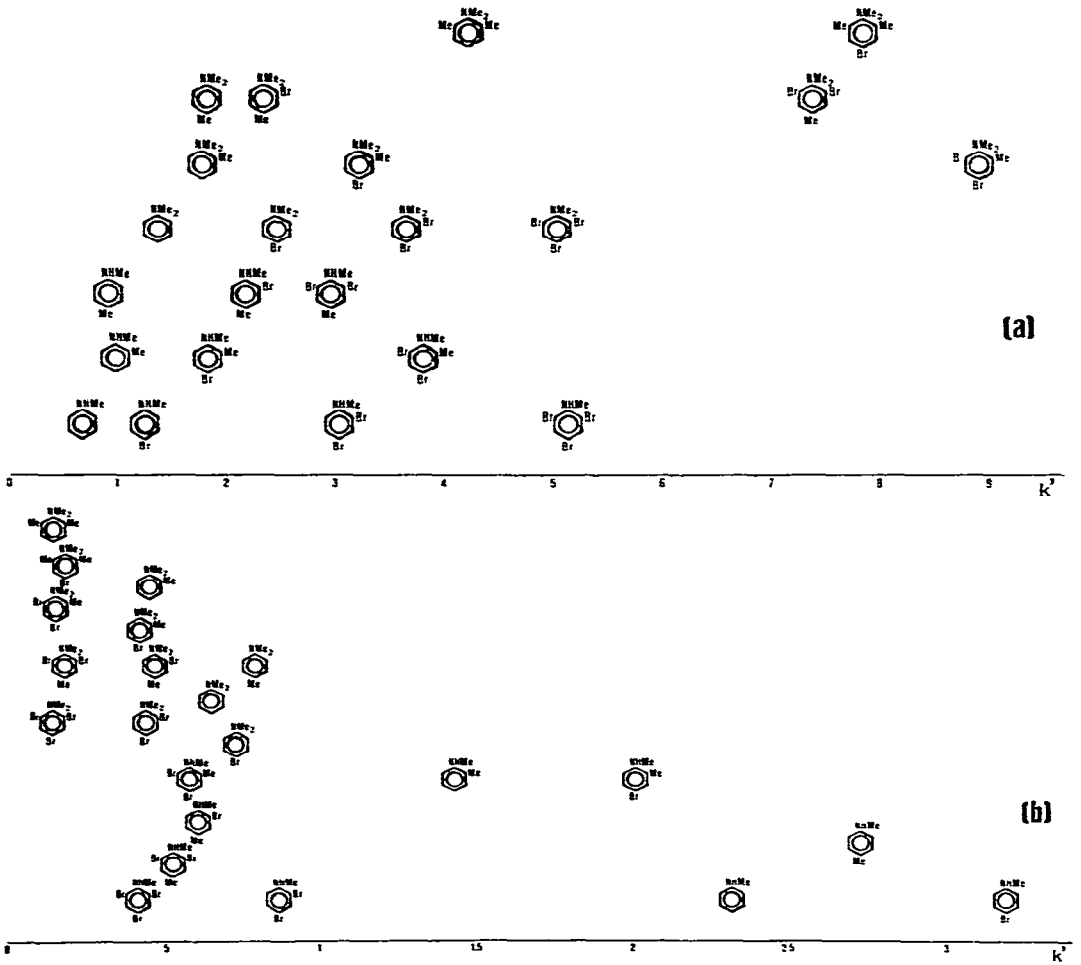

Fig. 6. Retention of secondary and tertiary anilines and the corresponding bromination products. (a) Reversed-phase LC. Column, Nucleosil C_{18}; eluent, methanol–aqueous buffer (80:20, v/v, pH 7.0), 45 ml h^{-1}. (b) Straight-phase LC. Column, Nucleosil CN; eluent, 0.2% (v/v) 2-propanol in isooctane, 45 ml h^{-1}.

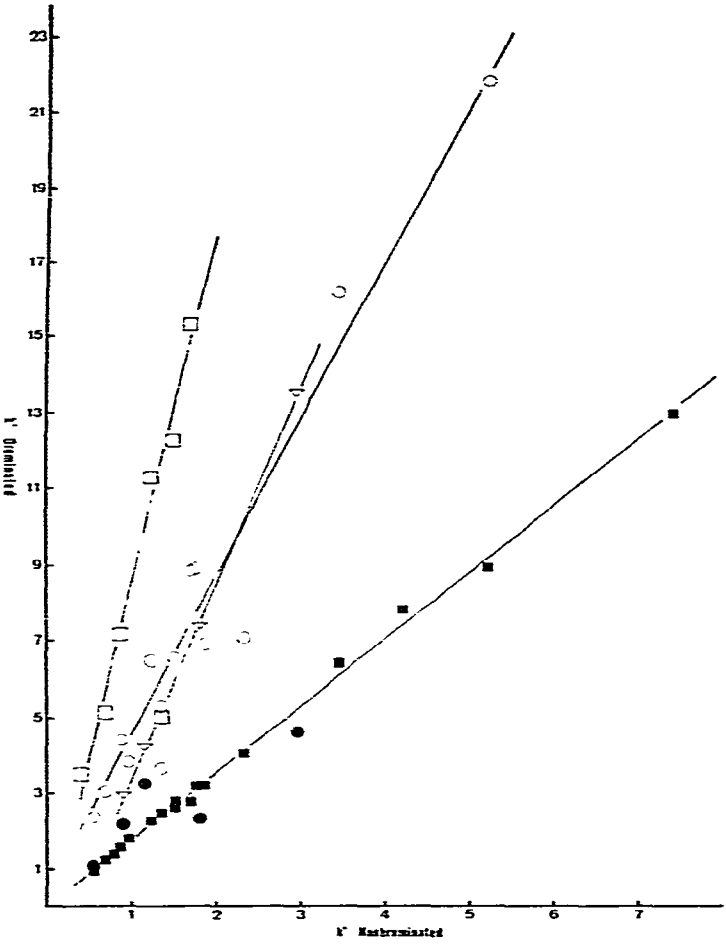

Fig. 7. Relationship between capacity factor, k', for alkylanilines and the corresponding bromination products in reversed-phase LC. Column, Nucleosil C_{18}; eluent, methanol–aqueous buffer (80:20, v/v, pH 7.0). 45 ml h^{-1}. ■ = Mono-*para*-brominated; ● = mono-*ortho*-brominated; ○ = *ortho*-*para*-brominated; ▽ = di-*ortho*-brominated; □ = tribrominated.

(a) Primary anilines. When bromine is substituted into aniline itself, or into a 2- or 4-alkylaniline, retention on the C_{18} phase increases with the number of bromine atoms entering. This is evident from the values of the separation factors, α, given in Table I. Among monobrominated anilines, the *para*-bromo-isomer is eluted before the corresponding *ortho*-bromo-isomer. Because *o*-bromoaniline, for example, is a weaker base than *p*-bromoaniline, it seems that the elution order is governed by the base strength, the least basic amine being eluted last. It is of interest to note that the same rule applies to *o*- and *p*-bromophenol on the C_{18} phase[17]. In this case *p*-bromophenol, which is the strongest acid, is eluted last. These facts are in accordance with the hydrophobic theory put forward by Horváth *et al.*[18] for the interaction between solutes and hydrocarbonaceous bonded stationary phases, using mixtures of water and organic solvents as mobile phases.

The retention effect of introducing bromine into a primary aniline is demonstrated in Fig. 8a and b, where the separation factors, α, for the monobrominated aniline and the aniline, respective the di- and mono-brominated anilines are plotted against carbon number. As can be seen, the increase in retention on mono-*ortho*-bromination is greater than on mono-*para*-bromination (Fig. 8a).

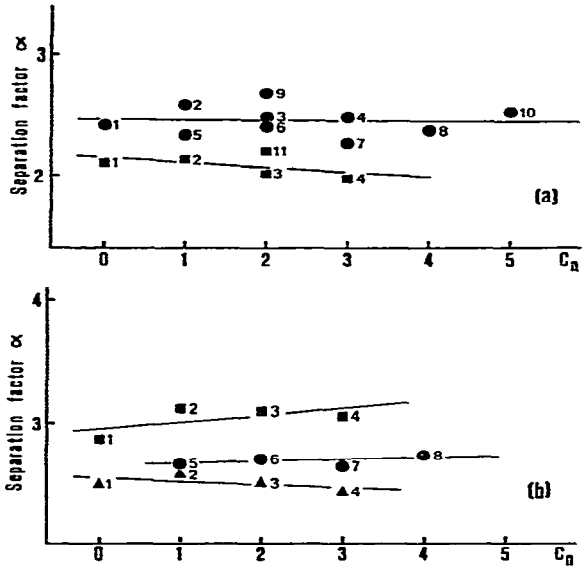

Fig. 8. Relationship between the separation factor, α, for monobrominated to non-brominated and for dibrominated to monobrominated primary alkylanilines, and alkyl carbon number. Column, Nucleosil C_{18}; eluent, methanol–aqueous buffer (70:30, v/v, pH 7.0), 45 ml h^{-1}. (a) Mono-/non-brominated primary alkylaniline: ■, *para*-/non-brominated; ●, *ortho*-/non-brominated. (b) Di-/monobrominated primary alkylaniline: ▲, *ortho-para*-/*ortho*-brominated; ●, di-*ortho*-/*ortho*-brominated; ■, *ortho-para*-/*para*-brominated. The numbers refer to Table I.

On further bromination of the originally formed *o*-bromoanilines, either *o,p*- or *o,o*-dibromoanilines are formed. As shown by Fig. 8b, the separation factors are slightly higher for the *o,o*-dibromoanilines, which is in accordance with the results from the monobromination. Dibromination of the *p*-bromoanilines yields only one product *viz.* *p,o*-dibromoanilines. In this case the separation factor for the di- and monobromination products is distinctly higher than for the above-mentioned series of dibrominated anilines.

The difference between, and relative constancy of, the separation factors for compounds formed on mono- and dibromination of primary anilines, makes it possible to predict the order of elution of bromination products formed on coulometric bromination of this kind of anilines, thereby facilitating the interpretation of reversed-phase chromatograms. The linear relationship between k' values of brominated and non-brominated primary anilines is also of value for this purpose.

The elution order on the C_{18} phase of aniline and some primary methylanilines and their bromination products is demonstrated in Fig. 5a, which also gives a further illustration of the retention rules previously discussed.

(b) Secondary and tertiary anilines. As for primary anilines, there exists a semi-linear relationship between k' values of non-brominated anilines and k' values of mono-, di- and tribrominated analogues, respectively (Fig. 7). However, it is obvious that certain compounds show a considerable deviation from a linear relationship.

The plot of separation factors against carbon numbers in Fig. 9a and b gives information about the retention change caused by the introduction of one, two and three bromine atoms, respectively, into a secondary aniline. As previously established for primary anilines, the increase in retention on mono-*ortho*-bromination is greater than that on mono-*para*-bromination (Fig. 9a). On further bromination of *o*-bromoanilines, *o,o*-dibromoanilines are formed. The increase in retention on this bromination is smaller than when a *p*-bromoaniline is brominated to a *p,o*-dibromoaniline, just as for the corresponding primary anilines. For the secondary *p,o*-dibromoanilines two different series can be discerned, *viz.* compounds with or without an *ortho*-situated alkyl group. The separation factors for the former series are lower and do not change with the carbon number. For the latter series the separation factor increases linearly with the carbon number, *i.e.* with the size of the N-alkyl group, when the first *ortho*-situated bromine atom is introduced, but remain constant

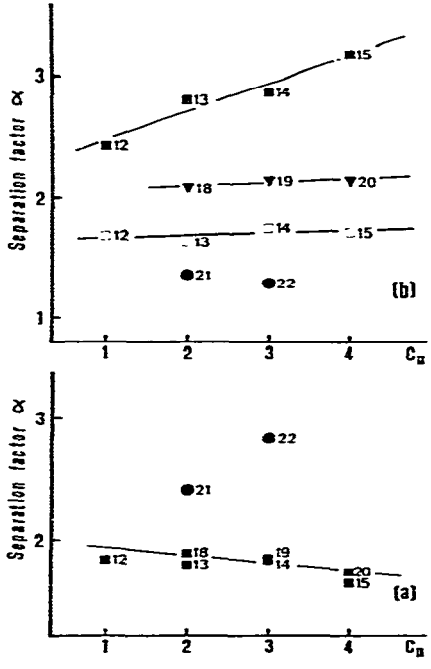

Fig. 9. Relationship between the separation factor, α, for monobrominated to non-brominated, dibrominated to monobrominated and tribrominated to dibrominated secondary alkylanilines, and alkyl carbon number. Column, Nucleosil C_{18}; eluent, methanol–aqueous buffer (80:20, v/v, pH 7.0), 45 ml h^{-1}. (a) Mono-, non-brominated secondary alkylaniline: ■, *para*-, non-brominated; ●, *ortho*-, non-brominated. (b) Di-, monobrominated and tri-, *ortho-para*-brominated secondary alkylanilines: ●, di-*ortho*-/*ortho*-brominated; ▼, *ortho-para*-/*para*-brominated (*ortho*-alkyl-substituted); ■, *ortho-para*-/*para*-brominated (non-*ortho*-alkyl-substituted); □, tri- *ortho-para*-brominated. The numbers refer to Table I.

when, on tribromination, the second *ortho*-standing bromine atom is substituted (Fig. 9b).

For brominated tertiary anilines the retention pattern on the C_{18} phase is somewhat different from that of primary and secondary anilines. Thus, for the latter two groups the greatest change in retention, on monobromination, occurred on formation of *o*-bromoanilines from anilines, and the smallest change, on dibromination, occurred on formation of *o,o*-dibromoanilines from *o*-bromoanilines. For tertiary anilines the reverse is true as shown by Fig. 10a and b. There is also a reversal of the separation factors for the two series formed on *ortho*-bromination of *p*-bromoanilines.

It has been stated by Locke[15] that for compounds of similar type, selectivity in reversed-phase chromatography is primarily determined by the eluent and that elution order is in the inverse order of solute solubilities in the moving phase. However, it is uncertain to what extent the actual anilines should be regarded as being of "similar type", and it is more likely that the disparity in screening and steric hindrance between different *ortho*-brominated anilines affects the dispersion forces acting upon the molecule as well as its hydrophobicity.

The elution order on the C_{18} phase of some secondary and tertiary anilines and their bromination products is demonstrated in Fig. 6a, which also gives a further illustration of the retention rules discussed above.

Straight-phase chromatography. In a previous work[7], it was shown that the

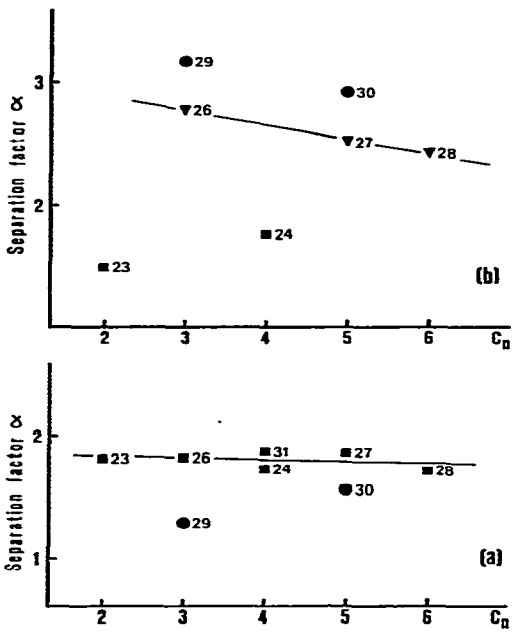

Fig. 10. Relationship between the separation factor, α, for monobrominated to non-brominated and dibrominated to monobrominated tertiary alkylanilines, and alkyl carbon number. Column, Nucleosil C_{18}; eluent, methanol–aqueous buffer (80:20, v/v, pH 7.0), 45 ml h^{-1}. (a) Mono-/non-brominated tertiary alkylaniline: ●, *ortho*-/non-brominated; ■, *para*-non/brominated. (b) Di-/monobrominated tertiary alkylaniline: ■, *ortho-para*-/*para*-brominated (non-*ortho*-alkyl-substituted); ▼, *ortho-para*-/*para*-brominated (*ortho*-alkyl-substituted); ●, di-*ortho*-/*ortho*-brominated. The numbers refer to Table I.

TABLE II

CAPACITY FACTORS AND SEPARATION FACTORS FOR *ortho*- AND *para*-ALKYL-SUBSTITUTED ANILINES AND THEIR BROMINATION PRODUCTS IN THE STRAIGHT-PHASE LC SYSTEM

Column, Nucleosil CN; eluent: 0.2% (v/v) 2-propanol in isooctane

No.	*Aniline (substituent)*	*Capacity factor, k'*						*Separation factor, α*					
		Non-brominated	*Monobrominated*		*Dibrominated*		*Tri-brominated*	*Mono-/non-*		*Di-/mono-ortho-*		*Di-/mono-para-*	*Tri-/di-*
			Ortho	*Para*	*Di-ortho*	*Ortho-para*		*Ortho*	*Para*	*Di-ortho*	*Ortho-para*	*Ortho-para*	
1	None	7.53	2.65	10.7		3.96	0.87	0.35	1.42		1.49	0.37	0.22
2	2-Methyl	4.84	1.41	6.74		2.06		0.29	1.39		1.46	0.31	
3	2-Ethyl	3.65	1.13	5.20		1.57		0.31	1.42		1.39	0.30	
4	2-Isopropyl	3.14	0.93	4.45		1.28		0.30	1.42		1.38	0.29	
5	4-Methyl	9.62	2.50		0.67			0.26		0.27			
6	4-Ethyl	8.72	2.26		0.60			0.26		0.27			
7	4-Isopropyl	8.87	2.16		0.57			0.24		0.26			
8	4-*n*-Butyl	8.72	2.11		0.54			0.24		0.26			
9	2,4-Dimethyl	6.04	1.36					0.23					
10	2-Methyl-4-butyl	5.07	1.20					0.24					
11	2,6-Dimethyl	2.83		3.91					1.38				
12	N-Methyl	2.32		3.19		0.87	0.42		1.38			0.27	0.48
13	N-Ethyl	1.49		1.98		0.51	0.29		1.33			0.26	0.57
14	N-Propyl	1.06		1.50		0.41	0.25		1.42			0.27	0.61
15	N-*n*-Butyl	0.90		1.27		0.37	0.23		1.41			0.29	0.62

16	N-Phenyl	2.50	0.61	6.89	0.87*	4.01**	1.04***	0.24	2.76				
17	N-Benzyl	1.65		2.62		1.43	0.43		1.59			0.55	0.30
18	N-Methyl-2-methyl	1.43		2.01		0.58			1.41			0.29	
19	N-Ethyl-2-methyl	0.82		1.11		0.39			1.35			0.35	
20	N-Ethyl-2-ethyl	0.67		0.90		0.32			1.34			0.36	
21	N-Methyl-4-methyl	2.73	0.61		0.53			0.22		0.87			
22	N-Ethyl-4-methyl	1.72	0.39		0.38			0.23		0.97			
23	N,N-Dimethyl	0.65		0.73		0.44	0.14		1.12			0.60	0.32
24	N,N-Diethyl	0.50		0.48		0.24			0.96			0.50	
25	N,N-Diphenyl	0.39		0.52		0.46**	0.41 ‡		1.33			0.88	0.89
26	N,N-Dimethyl-2-methyl	0.45		0.42		0.15			0.93			0.36	
27	N,N-Diethyl-2-methyl	0.29		0.22		0.13			0.76			0.59	
28	N,N-Diethyl-2-ethyl	0.21		0.18		0.11			0.86			0.61	
29	N,N-Dimethyl-4-methyl	0.79	0.47		0.18			0.59		0.38			
30	N,N-Diethyl-4-methyl	0.90	0.24		0.15			0.27		0.63			
31	N,N-Dimethyl-2,6-dimethyl	0.14		0.18					1.29				

* 2,4′-Dibrominated.
** 4,4′-Dibrominated.
*** 2,4,4′-Tribrominated; 2,4,2′,4′-tetrabrominated; $k' = 0.44$.
‡ 4,4′,4″-Tribrominated.

elution order on the nitrile phase of different kinds of anilines is tertiary, secondary and primary anilines and that the retention within each group is mainly determined by the number and size of *ortho*-situated alkyl groups and by alkyl groups substituted at the nitrogen atom.

The introduction of bromine into the aniline nucleus changes the elution order, and it is no longer possible to decide straight off, on the basis of k' values, if a brominated aniline is primary, secondary or tertiary. This fact is evident from the k' values presented in Table II. However, it can be seen that within each subgroup of brominated anilines, *i.e.* mono-*ortho*-bromo, mono-*para*-bromo-, etc., the k' values are to a great extent dissimilar and often allow the distinction between primary, secondary and tertiary bromoanilines of different kinds (Table III).

TABLE III

COMPARISON OF CAPACITY FACTORS, k', FOR BROMINATED PRIMARY, SECONDARY AND TERTIARY ALKYLANILINES IN THE STRAIGHT-PHASE LC SYSTEM

Column, Nucleosil CN; eluent: 0.2% (v/v) 2-propanol in isooctane.

Substituent	k'		
	Primary anilines	*Secondary anilines*	*Tertiary anilines*
Ortho-bromo	0.93– 2.65	0.39–0.61	0.24–0.47
Para-bromo	4.45–10.7	0.90–3.19	0.18–0.73
Di-*ortho*-bromo	0.54– 0.67	0.38–0.53	0.15–0.18
Ortho-para-dibromo	1.28– 3.96	0.32–0.87	0.11–0.44
Tribromo	0.87	0.23–0.42	0.14

The most striking nitrile phase retention effect of the introduction of bromine into an aniline is the great decrease in retention (with some exceptions) on *ortho*-bromination. This effect is primarily thought to be caused by steric hindrance due to the bromine atom, which restricts the interaction between the amino group and the nitrile group in the bonded phase. The substitution of bromine into the *para*-position of an aniline leads to either an increase in retention or to a small decrease, depending on the kind of aniline. This fact is further illustrated in Figs. 5b and 6b, which give the elution order on the nitrile phase of aniline and some methylanilines and their bromination products.

The semi-linear relationship between k' values of brominated and original anilines, previously established for the C_{18} phase, is also valid for the nitrile phase (Fig. 11). However, because of the increased selectivity of the nitrile phase, *o*-bromo- and *p*-bromoanilines fall on different lines and the same is true for *o,o*-dibromo- and *o,p*-dibromoanilines. The diagram in Fig. 11 is thus considerably more structurally informative than the corresponding diagram for the C_{18} phase in Fig. 7.

(a) Primary anilines. In Fig. 12a and b the separation factors for the brominated primary anilines on the nitrile phase are plotted against carbon numbers. The great difference in retention behaviour on *ortho*- and *para*-bromination, respectively, is clearly demonstrated.

(b) Secondary anilines. Mono-*ortho*- and mono-*para*-bromination of secondary anilines give rise to a similar separation factor picture as for primary anilines (Fig.

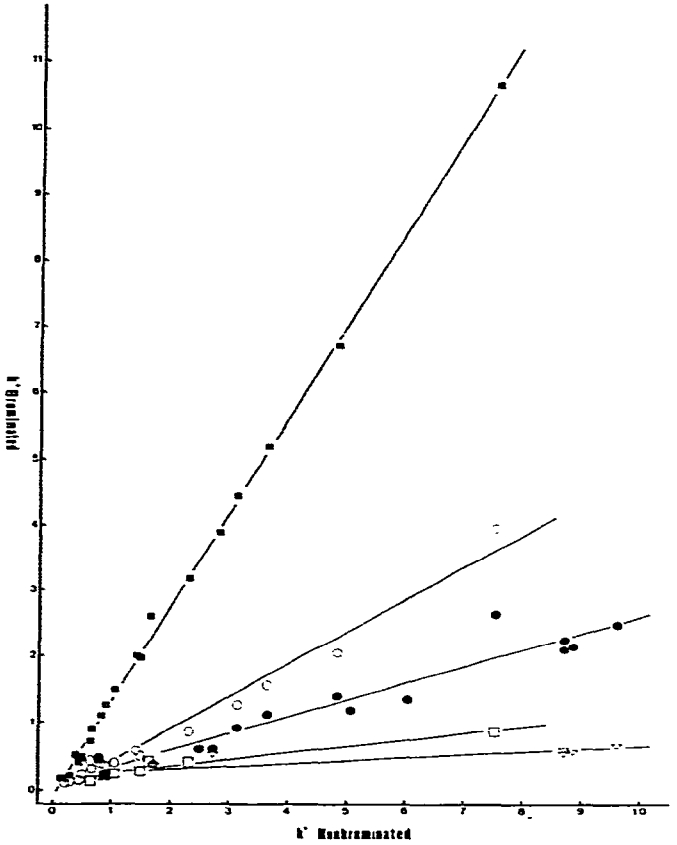

Fig. 11. Relationship between capacity factor, k', for alkylanilines and the corresponding bromination products in straight-phase LC. Column; Nucleosil CN; eluent, 0.2% (v/v) 2-propanol in isooctane, 45 ml h^{-1}. ■ = Mono-*para*-brominated; ● = mono-*ortho*-brominated; ○ = *ortho*-*para*-brominated; ▽ = di-*ortho*-brominated; □ = tribrominated.

13a). However, on introduction of a second and a third bromine atom the picture changes (Fig. 13b). While the lines for the conversion of *o*-bromo- to *o,o*-dibromo- and *p*-bromo- to *p,o*-dibromoanilines coincide for primary anilines, they are well separated for secondary anilines, and the retention change is far less for the former series. Hence, when 2-bromo-4-methyl-N-ethylaniline (No. 22) is brominated to 2,6-dibromo-4-methyl-N-ethylaniline the decrease in retention is very small.

The same tendency to a diminished change in retention on *ortho*-bromination of a secondary *o*-bromoaniline is shown on *ortho*-bromination of secondary *o,p*-dibromoanilines. Thus, when a third bromine atom is introduced into the *ortho*-position of these compounds the decrease in retention is considerably less than when 2,4-dibromoaniline is brominated to 2,4,6-tribromoaniline (Table II).

(c) Tertiary anilines. Most notable on bromination of tertiary anilines, is the behaviour on mono-*para*-bromination. On mono-*para*-bromination of primary and secondary anilines, *p*-bromoanilines with a greater retention than the original aniline resulted, but mono-*para*-bromination of tertiary anilines produced *p*-bromoanilines

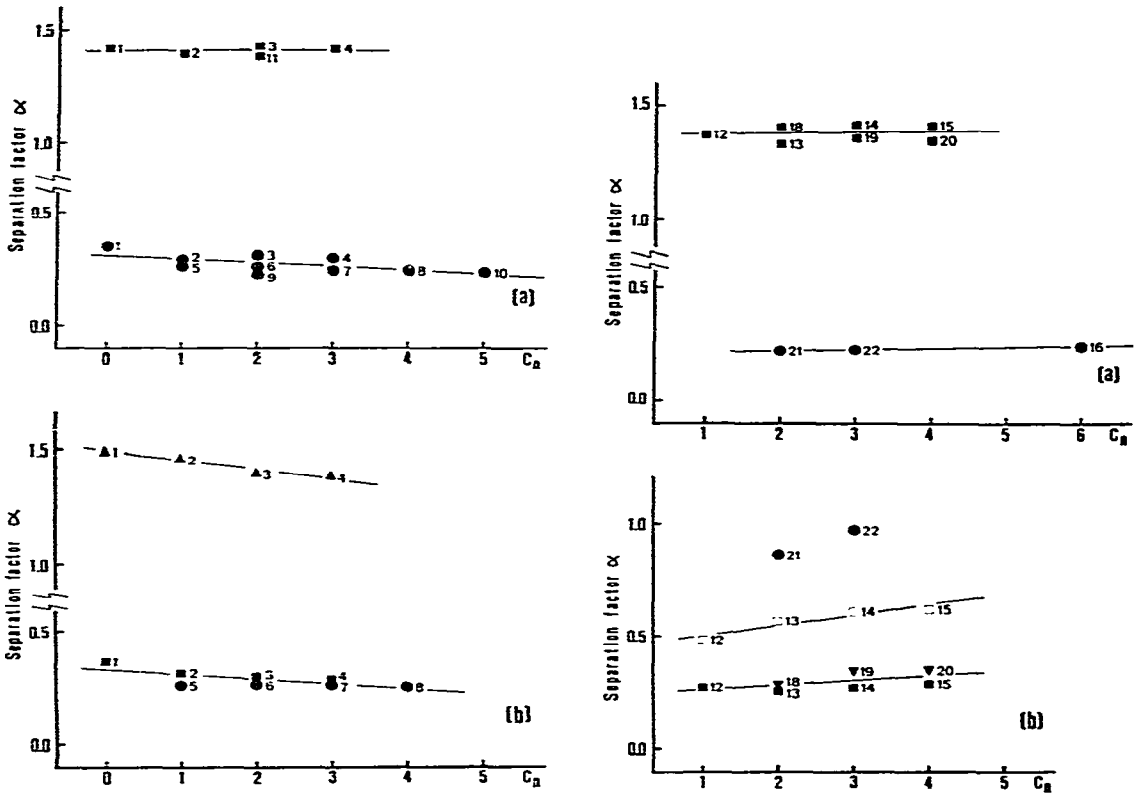

Fig. 12. Relationship between the separation factor, α, for monobrominated to non-brominated and dibrominated to monobrominated primary alkylanilines, and alkyl carbon number. Column, Nucleosil CN; eluent, 0.2% (v/v) 2-propanol in isooctane, 45 ml h^{-1}. (a) Mono-/non-brominated primary alkylaniline: ●, *ortho*-/non-brominated; ■, *para*-/non-brominated. (b) Di-/monobrominated primary alkylaniline: ●, di-*ortho*/*ortho*-brominated; ■, *ortho*-*para*-/*para*-brominated; ▲, *ortho*-*para*-/*ortho*-brominated. The numbers refer to Table II.

Fig. 13. Relationship between the separation factor, α, for monobrominated to non-brominated, dibrominated to monobrominated and tribrominated to dibrominated secondary alkylanilines, and alkyl carbon number. Column, Nucleosil CN; eluent, 0.2% (v/v) 2-propanol in isooctane, 45 ml h^{-1}. (a) Mono-/non-brominated secondary alkylaniline: ●, *ortho*-/non-brominated; ■, *para*-/non-brominated. (b) Di-/monobromined and tri-/*ortho*-*para*-brominated secondary alkylanilines: ●, di-*ortho*-/*ortho*-brominated; ■, *ortho*-*para*-/*para*-brominated (non-*ortho*-alkyl-substituted); ▼, *ortho*-*para*-/*para*-brominated (*ortho*-alkyl-substituted); □, tri-/*ortho*-*para*-brominated. The numbers refer to Table II.

which travelled either more slowly or more rapidly than the original aniline (Fig. 14a). The reason for this is discussed below.

Causes of retention change on bromination. In a previous study on retention behaviour of alkylanilines in straight-phase chromatography it was shown that retention was mainly governed by base strength and by the substitution pattern around the nitrogen atom[7]. This is partly true also for bromoanilines. Thus, the decrease in retention following *ortho*-bromination is considered to be due to a decrease in base strength and to increased steric hindrance to interaction between the amino group and the bonded-phase nitrile group.

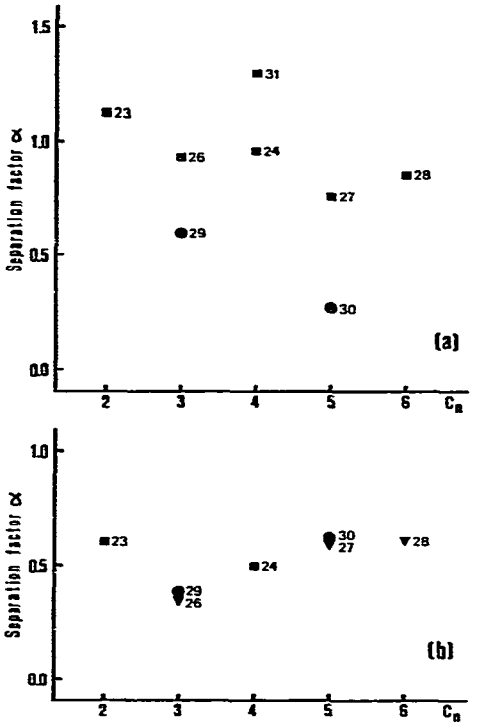

Fig. 14. Relationship between the separation factor, α, for monobrominated to non-brominated and dibrominated to monobrominated tertiary alkylanilines, and alkyl carbon number. Column, Nucleosil CN; eluent, 0.2% (v/v) 2-propanol in isooctane, 45 ml h^{-1}. (a) Mono-/non-brominated tertiary alkylaniline: ●, *ortho*-/non-brominated; ■, *para*-/non-brominated. (b) Di-/monobrominated tertiary alkylaniline: ●, di-*ortho*-/*ortho*-brominated; ■, *ortho*-*para*-/*para*-brominated (non-*ortho*-alkyl-substituted); ▼, *ortho*-*para*-/*para*-brominated (*ortho*-alkyl-substituted). The numbers refer to Table II.

The fact that *para*-bromination generally causes an increase in retention cannot be explained in this way. Since the environment of the nitrogen atom is not changed on *para*-bromination and the base strength decreases, one would rather expect the retention to decrease on *para*-bromination. There is obviously a third factor which influences retention of *p*-bromoanilines. It is suggested that this factor is a decreased solubility of the *p*-bromoaniline in the mobile phase in comparison with the original aniline, causing it to travel more slowly than this compound.

Among *para*-bromo-substituted tertiary anilines some compounds were eluted before the original aniline (Fig. 14a). This deviation from the general retention rule for *p*-bromoaniline is most likely a result of the interplay between the change in base strength and the change in solubility on bromination. In this case the decrease in solubility is not great enough to match the decrease in base strength, which causes the *p*-bromoaniline to travel more rapidly than the original aniline.

Although the base strength of the aniline, and especially steric effects, were considered mainly to govern retention change on *ortho*-bromination, solvent effects cannot wholly be left out of consideration. Thus, the different retention behaviour of certain secondary anilines on *ortho*-bromination compared to corresponding primary and tertiary anilines (Fig. 13b) may well be caused by solubility differences.

CONCLUSIONS

For the investigated anilines the coulometric bromination technique described by Truedsson and Smith[1] yields predictable, unambiguous 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*-bromoanilines.

Over-bromination of primary anilines leads to oxidation products, especially for *ortho*-alkyl-substituted compounds, whereas *para*-alkyl-substituted primary anilines show a far better stability against excess of bromine. The secondary and tertiary anilines studied in this work are generally not fully brominated at the end-point, but still contain vacant *ortho*-positions. On over-bromination, these free positions are substituted with bromine and, on further bromination, loss of N-alkyl groups occurs with the formation of primary and secondary anilines.

Introduction of bromine into the *ortho*- and *para*-positions of an alkylaniline causes an increase in retention in the reversed-phase LC system. There is a semi-linear relationship between k' values of brominated and non-brominated anilines, which is of value for identification purposes.

In the straight-phase LC system, *para*-bromination generally causes an increase in retention and *ortho*-bromination a decrease. As for the reversed-phase system, there is a semi-linear relationship between k' values of brominated and original anilines.

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