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SEPARATION OF METHYL-SUBSTITUTED BENZ[*c*]ACRIDINES BY GAS–LIQUID CHROMATOGRAPHY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

KUNIHIRO KAMATA*
Metropolitan Research Laboratory of Public Health, 24-1, Hyakunincho 3-chome, Shinjuku-Ku, Tokyo, 160 (Japan)
and
NOBORU MOTOHASHI
Meiji College of Pharmacy, Yato-cho, Tanashi, Tokyo, 188 (Japan)
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SUMMARY

The separation of methyl-substituted benz[*c*]acridines (BAc) was investigated by gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC). GLC separations were carried out on a glass column packed with Chromosorb W AW DMCS (80–100 mesh) coated with various types silicone stationary phases. For HPLC separations both reversed-phase and normal-phase techniques were used with various mobile phases. Non-polar stationary-phase GLC made possible the separation of BAc, according to the number of carbon atoms, which little selectivity toward isomers. In polar stationary-phase GLC and normal-phase HPLC, a steric hindrance between the nitrogen atom and some substituents of the BAc ring apparently influenced the retention time of BAc.

INTRODUCTION

Recently, aza-arenes have been extensively studied because of their carcinogenic[1–3] and mutagenic properties. Aza-arenes can occur in diverse sources such as atmospheric matters[4,5], tobacco smoke[6–9] and automobile exhaust[10]. They are also present in high boiling petroleum distillates[11] and constitute substantial fractions of shale oil[12], coal tar[9,13] and coal liquefaction products[14,15].

It is important, therefore, to identify and determine aza-arenes. Unfortunately, there is little information on the chromatography of methyl-substituted benz[*c*]acridines (BAc), which are known to show strong carcinogenicity[16]. BAc have been analysed by means of paper chromatography[17] or thin-layer chromatography[18,19]. There are few reports on the separation of BAc by gas–liquid chromatography (GLC)[19–21] or high-performance liquid chromatography (HPLC)[14,22].

In this paper, the GLC and HPLC separation and retention patterns of twelve BAc will be examined.

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EXPERIMENTAL

Materials

The twelve BAc were synthesized according to the literature[23–29] and purified as described in a previous paper[30]; benz[*c*]acridine (1), 7-methylbenz[*c*]acridine (2), 8-methylbenz[*c*]acridine (3), 9-methylbenz[*c*]acridine (4), 10-methylbenz[*c*]acridine (5), 11-methylbenz[*c*]acridine (6), 5,7-dimethylbenz[*c*]acridine (7), 7,9-dimethylbenz[*c*]acridine (8), 7,10-dimethylbenz[*c*]acridine (9), 7,11-dimethylbenz[*c*]acridine (10), 7,9,10-trimethylbenz[*c*]acridine (11) and 7,9,11-trimethylbenz[*c*]acridine (12). Both stock and standard solutions in ethanol were freshly prepared before use.

The reagents and solvents used for HPLC were obtained from Wako Pure Chemical (Osaka, Japan).

GLC

GLC of BAc was carried out with a Shimadzu Model 5A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector. The results were evaluated with the Shimadzu Chromatopac C-R1A digital integrator. The flow-rate of the nitrogen carrier gas was maintained at 60 ml/min, air flow-rate at 100 ml/min and hydrogen flow-rate at 50 ml/min, respectively. The sample injection volume was 1 μl of an ethanolic solution containing 0.5 μg of each BAc. The temperatures of the column and injection port were 220–240 and 290°C, respectively. The stationary phases 2% OV-1, 2% OV-7, 2% OV-17, 2% OV-210, 2% OV-225, 2% OV-275 and 5% XE-60 stationary phases coated on Chromosorb W AW DMCS (80–100 mesh) as support were packed in 2 m and 1.5 m × 3 mm I.D. glass columns.

HPLC

The HPLC apparatus consisted of a JASCO pump Model Trirotar-II, a Rheodyne injector Model 7125 equipped with 20-μl loop and a JASCO spectrophotometer Model UVIDEC-100 III. Prepacked LiChrosorb RP-8 (7 μm, 250 × 4.6 mm I.D.; Cica Merck), LiChrosorb RP-18 (10 μm; 250 × 4.6 mm I.D.; Cica Merck), LiChrosorb RP-18 (5 μm, 250 × 4.6 mm I.D.; Cica Merck), Zorbax C_{18} (150 × 4.6 mm I.D.; DuPont), Zorbax SIL (250 × 4.6 mm I.D.; DuPont) and LiChrosorb NH_2 (5 μm, 250 × 4.6 mm I.D.; Cica Merck) were used. All the solvents were filtered through Millipore membrane filters (0.45 μm) and degassed under vacuum prior to use. The mobile phase compositions are given in volume percentages. The sample injected was 5 μl of an ethanolic solution containing 10 ng of each BAc. BAc were detected by monitoring the UV absorbance of the column eluate at 280 nm. The results were evaluated by the Shimadzu Chromatopac C-R1A digital integrator.

RESULTS AND DISCUSSION

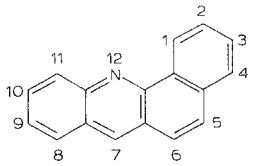
The structures and carcinogenic activities[16] of BAc ware given in Table I.

GLC of BAc

Changing the polarity of the stationary phase has an important effect on the separation of aza-arenes[21]. Schmitter *et al.*[31] determined the retention indices of aza-arenes on a non-polar, medium-polar and polar types of stationary phases. They

TABLE I

STRUCTURES AND CARCINOGENIC ACTIVITIES OF BENZ[c]ACRIDINES



Compd. No.	*Carcinogenic activity**	*Name*
1	−	Benz[*c*]acridine
2	+	7-Methylbenz[*c*]acridine
3	−	8-Methylbenz[*c*]acridine
4	−	9-Methylbenz[*c*]acridine
5	−	10-Methylbenz[*c*]acridine
6	−	11-Methylbenz[*c*]acridine
7	+	5,7-Dimethylbenz[*c*]acridine
8	+	7,9-Dimethylbenz[*c*]acridine
9	+	7,10-Dimethylbenz[*c*]acridine
10	+	7,11-Dimethylbenz[*c*]acridine
11	+	7,9,10-Trimethylbenz[*c*]acridine
12	+	7,9,11-Trimethylbenz[*c*]acridine

* Skin tumour-producing activity: +, active; −, inactive.

suggested that the prediction of the retention sequence for aza-arene isomers would be very difficult on polar stationary phases.

The GLC behaviour of twelve BAc was examined on seven stationary phases, that is OV-1, OV-7, OV-17, OV-210, OV-225, OV-275 and XE-60. The retention times of BAc relative to compound 1 are shown in Table II. Symmetric peaks were obtained for all twelve BAc. On OV-225 and XE-60 the separation patterns were very similar. While none of the seven stationary phases completely separated the BAc, combinations of the stationary phases were useful. For instance, compounds 1 and 6, 2 and 10, 8 and 12 and 9 and 12 could not be separated on both OV-225 and XE-60, but could be separated on both OV-1 and OV-275. Compounds 3 and 10 could not be separated on OV-275, but could be separated by using other stationary phases, and compounds 3 and 4, 3 and 5, 7 and 8, and 7 and 9 which could not be separated on OV-1, OV-7, OV-17 and OV-275 could be satisfactorily separated on OV-275. On the other hand, compounds 4 and 5, and 8 and 9 could not be separated in using seven stationary phases.

The relationship between the retention time and the position of the methyl groups in BAc and the polarity of the stationary phases was evaluated in terms of the McReynolds' constant[32], Fig. 1 ($\Sigma\Delta I$ = 222 for OV-1, 592 for OV-7, 884 for OV-17, 1500 for OV-210, 1813 for OV-225 and 4938 for OV-275). The elution order of BAc on a non-polar stationary phase (OV-1) followed that of the order of increasing number of carbon atoms. But, the retention behavior of compounds substituted by a methyl group at position 11 apparently differed from those of the other deriv-

TABLE II

GLC RETENTION DATA OF BAc ON SEVEN KINDS OF STATIONARY PHASES

Relative to compound 1 = 1.00. Compounds as in Table I.

Compd. No.	*Stationary phase, column length and column temperature*						
	2% OV-1 2 m 220°C	*2% OV-7 2 m 240°C*	*2% OV-17 2 m 240°C*	*2% OV-210 2 m 220°C*	*2% OV-225 2 m 240°C*	*2% OV-275 1.5 m 220°C*	*5% XE-60 1 m 240°C*
1	1.00 (1.7 min)	1.00 (6.5 min)	1.00 (3.0 min)	1.00 (3.7 min)	1.00 (16.2 min)	1.00 (5.7 min)	1.00 (3.9 min)
2	1.61	1.66	1.70	1.58	1.72	1.66	1.73
3	1.42	1.41	1.41	1.44	1.45	1.35	1.48
4	1.35	1.37	1.35	1.39	1.34	1.20	1.39
5	1.35	1.37	1.36	1.37	1.35	1.22	1.36
6	1.19	1.15	1.11	1.07	1.04	0.83	1.05
7	2.21	2.30	2.31	2.27	2.37	2.10	2.49
8	2.13	2.22	2.19	2.22	2.23	1.96	2.32
9	2.21	2.23	2.22	2.17	2.26	1.96	2.39
10	1.87	1.89	1.84	1.64	1.72	1.32	1.84
11	3.28	3.47	3.48	3.48	3.57	3.04	3.95
12	2.47	2.48	2.37	2.15	2.19	1.50	2.45

atives on polar stationary phases. This effect which can be related to the steric hindrance of substituents located near the nitrogen atom, was similar to that proposed by Schmitter *et al.*[31].

At the same temperature, retention times measured on OV-1 and OV-275 are equal. Fig. 2 shows that the compounds fall into one of two types, those lying on the

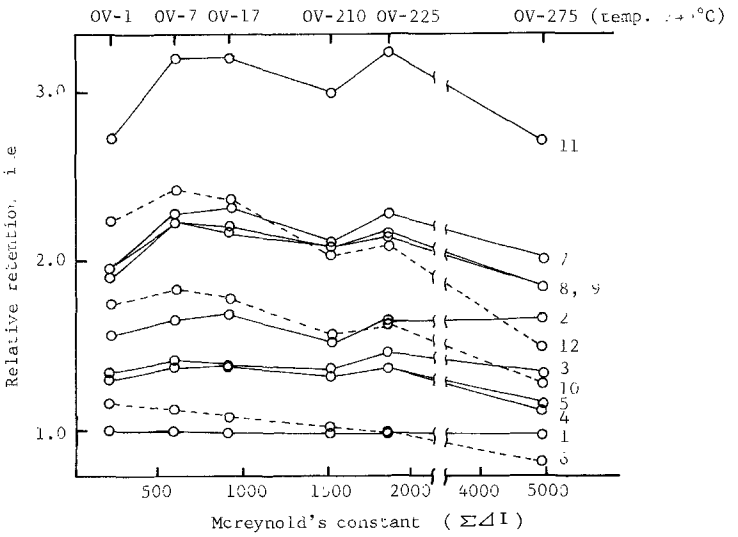


Fig. 1. Relationships between McReynolds' constant and the retention behaviour of BAc relative to compound 1 (1.00).

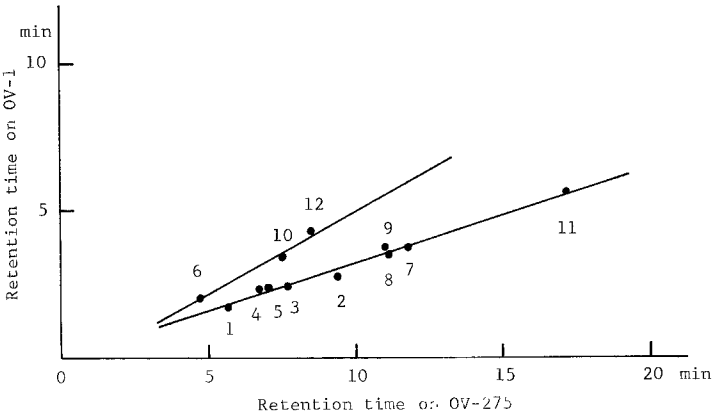
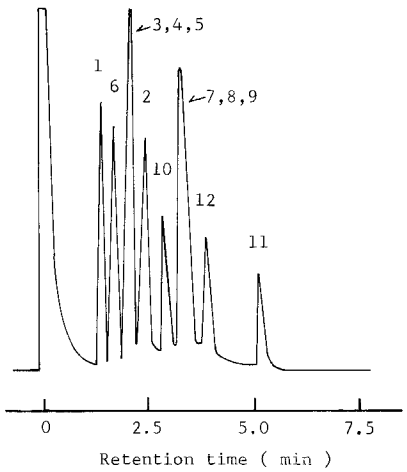


Fig. 2. Relationships between the retention times of BAc on OV-1 and OV-275 columns at 220°C (column temperature).

upper line having a methyl substituent at position 11 (type I), while those on the lower line had no methyl substituent in this position (type II). A linear regression of the upper line made with the retention data of compounds 6, 10 and 12 gives a slope of 0.541, an intercept of 0.587 and a regression coefficient of 0.974, and for the lower line with the retention data of compounds 1, 2, 3, 4, 5, 7, 8, 9 and 11 gives 0.328, 0.062 and 0.993, respectively. This result could be explained in terms of a specific interaction between the eluites and the stationary phases. When the polarity of the stationary phase increased, the interaction of BAc with the polar stationary phase was generally enhanced relative to the compounds of type I exhibiting steric hindrance of the nitrogen atom.

It was concluded that the elution sequence of BAc on non-polar silicone stationary phases follows the order of increasing number of carbon atoms, but the prediction of the retention sequence is very difficult for polar stationary phases. The



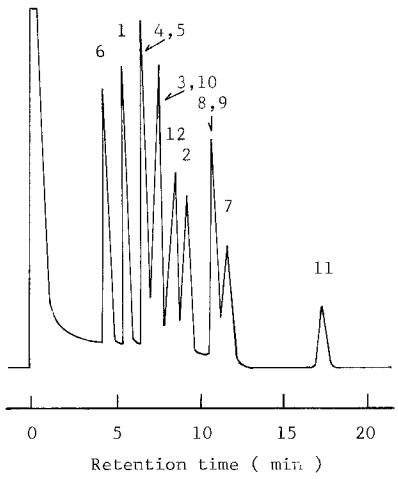


Fig. 3. Gas chromatogram of the separation of twelve BAc on OV-1 column.

Fig. 4. Gas chromatogram of the separation of twelve BAc on OV-275 column.

TABLE III

HPLC RETENTION DATA OF BAc

Mobile phases: A, methanol–water (8:2); B, acetonitrile–water (6:4); C, methanol–water (9:1); D, acetonitrile–water (8:2); E, methanol–water (9:1) containing 0.002 *M* 1-pentanesulphonic acid sodium salt; F, acetonitrile–water (8:2) containing 0.002 *M* 1-pentanesulphonic acid sodium salt; G, methanol–water (85:15) containing 0.002 *M* 1-pentanesulphonic acid sodium salt; H, acetonitrile–water (7:3) containing 0.002 *M* 1-pentanesulphonic acid sodium salt; I, ethanol–hexane (5:95); J, hexane. Compounds as in Table I. Relative to compound 1 = 1.00.

Compd. No.	*LiChrosorb RP-8 (7 μm)*		*LiChrosorb RP-18 (10 μm)*		*LiChrosorb RP-18 (5 μm)*		*Zorbax C_{18}*		*Zorbax SIL I (1.5 ml/min)*	*LiChrosorb NH_2 (5 μm) J (1 ml/min)*
	A (1 ml/min)	*B (1 ml/min)*	*C (1 ml/min)*	*D (1 ml/min)*	*E (0.5 ml/min)*	*F (0.5 ml/min)*	*G (1 ml/min)*	*H (1 ml/min)*		
1	1.00 (10.7 min)	1.00 (13.5 min)	1.00 (7.9 min)	1.00 (9.2 min)	1.00 (15.3 min)	1.00 (19.8 min)	1.00 (7.8 min)	1.00 (10.8 min)	1.00 (4.0 min)	1.00 (17.2 min)
2	1.24	1.17	1.24	1.20	1.25	1.22	1.33	1.29	2.10	1.13
3	1.24	1.24	1.24	1.24	1.25	1.23	1.35	1.34	1.11	0.98
4	1.26	1.26	1.29	1.35	1.32	1.37	1.45	1.48	1.11	1.02
5	1.22	1.23	1.22	1.30	1.26	1.30	1.36	1.41	1.56	1.06
6	1.88	1.83	1.79	1.76	1.82	1.77	2.29	2.21	0.61	0.54
7	1.65	1.50	1.62	1.54	1.64	1.59	1.90	1.81	2.23	1.12
8	1.57	1.47	1.57	1.56	1.59	1.61	1.86	1.82	2.88	1.24
9	1.57	1.45	1.57	1.57	1.58	1.61	1.84	1.84	4.70	1.25
10	2.34	2.15	2.27	2.16	2.34	2.20	3.19	2.85	0.61	0.56
11	1.99	1.72	1.97	1.90	1.96	2.01	2.47	2.41	8.13	1.48
12	3.11	2.78	3.10	2.90	3.20	3.00	4.84	4.28	0.61	0.57

excellent separations between closely related BAc isomers were obtained by the combination of a non-polar (OV-1) and polar (OV-275) stationary phases (Figs. 3 and 4); on the other hand, medium-polar stationary phases did not yield a good separation.

HPLC of BAc

Since HPLC is more rapid and more simple than GLC, its use for aza-arenes has recently been reported[33–35]. As the polarity of BAc was shown to be very weak, the proper selection of a mobile phase and a stationary phase was important to achieve a clear HPLC separation of BAc.

HPLC of BAc was done by using both reversed-phase and normal-phase types of column and various mobile phases. The chromatographic behaviour of BAc is shown in Table III, relative to the retention of compound 1.

Several parameters modify the separation of BAc in reversed-phase liquid chromatography (RPLC): (1) the nature of the stationary phases (the length of the alkyl chain, pore size), (2) the composition of the mobile phase (the water content, pH value and the concentration of different species for ion pairing, etc.) and (3) the temperature.

The solvent had a very strong influence on the quality of the HPLC separation, especially for the more polar BAc. The mobile phases chosen for BAc were methanol–water and acetonitrile–water. HPLC patterns of a mixture of twelve BAc obtained by using two mobile phases and either a LiChrosorb RP-8 or a LiChrosorb RP-18 (10 μm) column are shown in Figs. 5 and 6. Some variations in the retention sequences are evident from a comparison of the elution behaviour of BAc with two mobile phases. However, chromatograms obtained on LiChrosorb RP-18 (5 μm) and Zorbax C_{18} showed very broad peaks for a few BAc. Fig. 7 shows the HPLC pattern of a mixture of twelve BAc obtained using two mobile phases on LiChrosorb RP-18 (5 μm). To obtain a better resolution an ion-pairing agent (1-pentanesulphonic acid sodium salt) was added to the mobile phase. HPLC patterns of BAc on LiChrosorb RP-18 (5 μm) and Zorbax C_{18} were very similar. Those obtained on Zorbax C_{18} with methanol–water or acetonitrile–water as mobile phases containing 1-pentane-

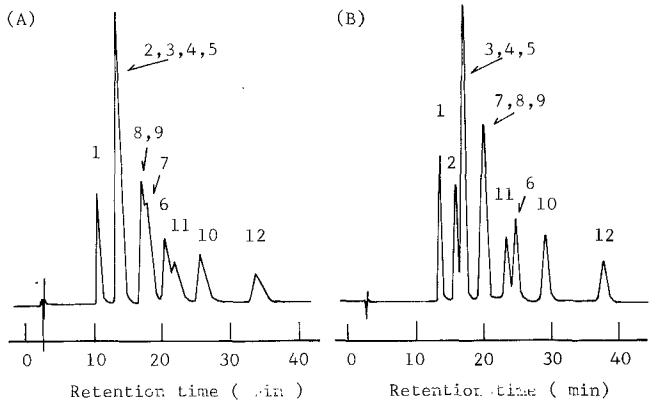


Fig. 5. Separation of twelve BAc on LiChrosorb RP-8 column. Mobile phases: A, methanol–water (8:2); B, acetonitrile–water (6:4).

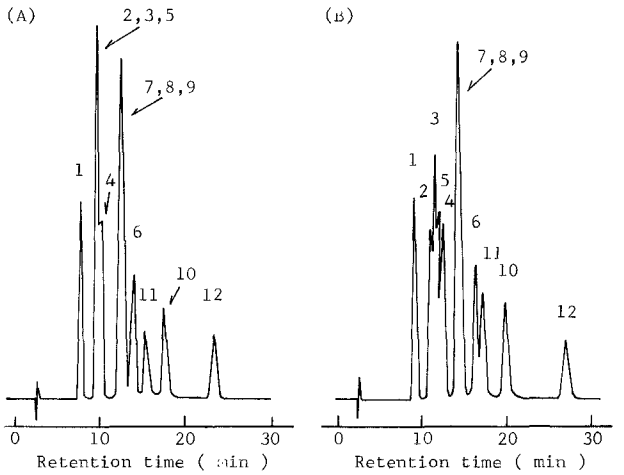


Fig. 6. Separation of twelve BAc on LiChrosorb RP-18 (10 μm) column. Mobile phases: A, methanol–water (9:1); B, acetonitrile–water (8:2).

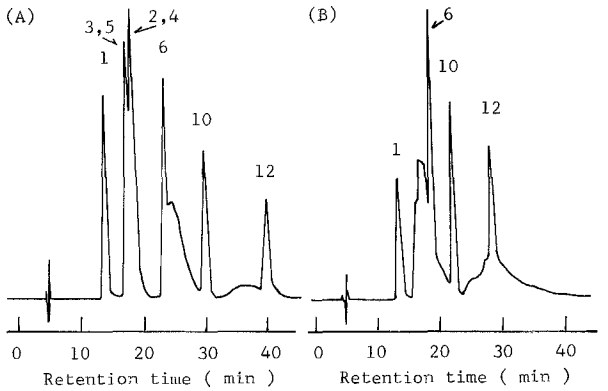


Fig. 7. Separation of twelve BAc on LiChrosorb RP-18 (5 μm) column. Mobile phases: A, methanol–water (9:1); B, acetonitrile–water (9:1).

sulphonic acid sodium salt are shown in Fig. 8. The separation of compounds 2, 3 and 5 was very clear in acetonitrile–water, but incomplete in methanol–water. The effect of the steric hindrance of the nitrogen atom on the retention time of BAc in HPLC may be important here, as was shown by GLC retention times. The general trend should be an increase in retention with increasing molecular weight, however, not all BAc showed this trend. Dimethyl derivatives (compounds 7–9) were eluted faster than monomethyl (6), and the trimethyl derivative (11) was eluted faster than the dimethyl (10). A clear separation between closely related isomers was obtained on LiChrosorb RP-18 (5 μm) and on Zorbax C_{18} when 1-pentanesulphonic acid sodium salt was added to the acetonitrile–water mobile phase. However, reversed-phase partition systems did not satisfactorily separate compounds 7–9 (Fig. 8).

Normal-phase liquid chromatography (NPLC) was particularly useful in sep-

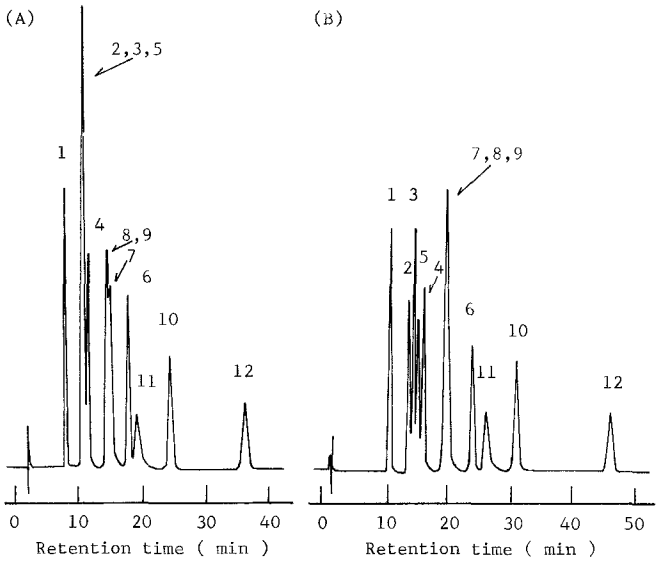
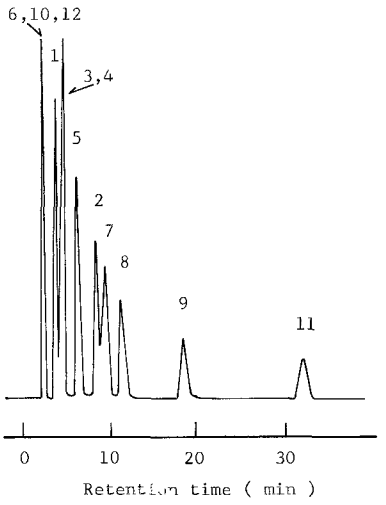


Fig. 8. Separation of twelve BAc on Zorbax C_{18} column. Mobile phases: A, methanol–water (85:15) containing 0.002 *M* 1-pentanesulphonic acid sodium salt; B, acetonitrile–water (7:3) containing 0.002 *M* 1-pentanesulphonic acid sodium salt.

arating compounds 7–9, which could not be sufficiently separated by RPLC (Table III). Studies have been conducted of the separation of BAc on Zorbax SIL and LiChrosorb NH_2. The separation on Zorbax SIL using ethanol–hexane as a mobile phase is shown in Fig. 9. The separation on LiChrosorb NH_2 column using hexane as a mobile phase is shown in Fig. 10. Compounds 6, 10 and 12 (type I) were eluted



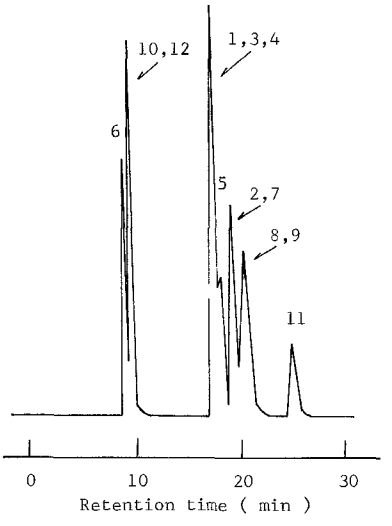


Fig. 9. Separation of twelve BAc on Zorbax SIL column.

Fig. 10. Separation of twelve BAc on LiChrosorb NH_2 column.

close to the solvent peak in the NPLC system; for these compounds the nitrogen atom would be most sterically hindered. Compounds 7–9, not distinguished by RPLC, were apparently separated on Zorbax SIL. On LiChrosorb NH_2, compounds 7 and 8, and 7 and 9 were clearly separated, but not compounds 8 and 9. Conversely, compounds 6, 10 and 12, which were separated by RPLC, could not be separated on Zorbax SIL.

It is suggested that BAc can be satisfactorily separated by HPLC.

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