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SEPARATION OF AZAARENES BY POLYAMIDE THIN-LAYER CHROMATOGRAPHY AND THE RELATIONSHIP BETWEEN THEIR BASICITY AND R_F VALUES

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SUMMARY

The separation of quinoline, isoquinoline, their methyl derivatives and the isomers of benzoquinolines and dibenzoquinolines was investigated by thin-layer chromatography using polyamide as adsorbent. When developed with neutral solvent systems, derivatives and isomers of azaarenes constructed of the same number of aromatic rings were hardly separated. However, using *n*-hexane–ethyl acetate–methanol–formic acid systems, separation was achieved due to differences in the basicity of the molecules caused by the effect of methyl groups and aromatic rings on the nitrogen atoms.

INTRODUCTION

Because a number of azaarenes formed under incomplete combustion of nitrogen-containing organic substances have been found to be carcinogenic in animals[1–3], as are polynuclear aromatic hydrocarbons, the concern of investigators has been focused on their presence in the environment. Several attempts have been made to detect azaarenes using paper partition chromatography[4,5] thin-layer chromatography (TLC)[5–8], column chromatography[6,9], high-performance liquid chromatography (HPLC)[10,11], gas–liquid chromatography (GLC) and GLC combined with mass spectrometry[10–13]. However, it is very important when approaching the problem of analysing azaarenes in environmental and biological samples to find techniques for separating the compounds from each other and for eliminating interfering materials with characteristics similar to those of azaarenes. Therefore, many of the

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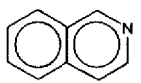
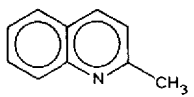
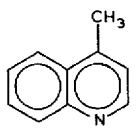
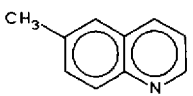
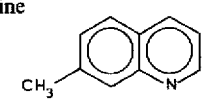
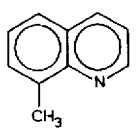
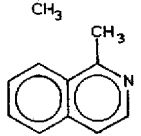
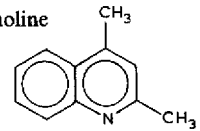
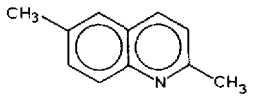
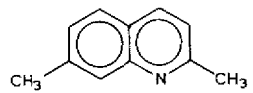
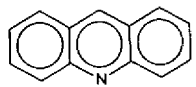
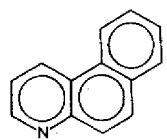
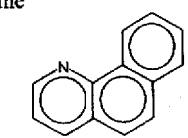
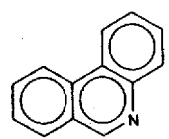
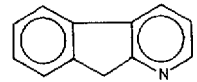
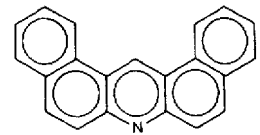
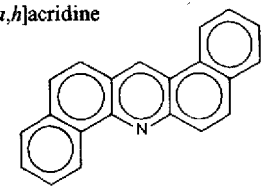
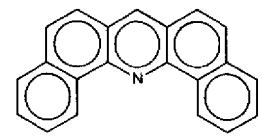
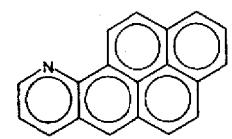
TABLE I

STRUCTURAL FORMULAE OF AZAARENES STUDIED

Compound No.	*Name*	*Formula*
1	Quinoline	
2	Isoquinoline	
3	2-Methylquinoline	
4	4-Methylquinoline	
5	6-Methylquinoline	
6	7-Methylquinoline	
	8-Methylquinoline	
8	1-Methylisoquinoline	
9	2,4-Dimethylquinoline	
10	2,6-Dimethylquinoline	
11	2,7-Dimethylquinoline	
12	Acridine	
13	Benzo[*f*]quinoline	
14	Benzo[*h*]quinoline	
15	Phenanthridine	
16	4-Azafluorene	
17	Dibenz[*a*,*j*]acridine	
18	Dibenz[*a*,*h*]acridine	
19	Dibenz[*c*,*h*]acridine	
20	10-Azabenzo[*a*]pyrene	

systems for analysing azaarenes have been based on their extraction and a combination of chromatographic techniques[6,7,10,11].

TLC is especially used as a final step in the analysis of azaarenes for the purposes of both detection and as a clean-up procedure for the elimination of interfering materials. For the former purpose, systems for separating two- to five-ring azaarenes using alumina[6,8], silica gel[9] and polyamide[5] as adsorbents have already been reported, showing that separation on silica gel is superior to that on alumina, and that on polyamide they are mainly distributed according to molecular weight. TLC systems giving separation patterns different to those already obtained would be useful for the detection and confirmation of azaarenes at low levels in the environment.

The main object of this investigation was to obtain, using polyamide plates, improved separations of azaarenes which might not be satisfactorily separated from each other by GLC or HPLC.

EXPERIMENTAL

Adsorbent

TLC alumina sheets [polyamide 11 F_{254} (20 × 20 cm)] were obtained from E. Merck (Darmstadt, G.F.R.).

Reagents

The azaarene compounds used in this study are listed in Table I. Dibenz[*a,j*]acridine, dibenz[*a,h*]acridine, dibenz[*c,h*]acridine and 10-azabenzo[*a*]pyrene were donated by Dr. K. Syudo (University of Tokyo, Tokyo, Japan). Other compounds were obtained from commercial sources. The test solutions of quinoline, isoquinoline and their methyl derivatives were prepared by dissolving in acetone to make 1% solutions, while those of 4-azafluorene, the four benzoquinolines, the three dibenzacridines and 10-azabenzo[*a*]pyrene were prepared by dissolving in acetone to make 0.2, 0.15, 0.01 and 0.01% solutions, respectively. All solvents were of analytical-reagent grade.

Apparatus

A chromatographic chamber (12 × 22 × 25 cm) equipped with a suspension unit devised for pre-equilibrating the thin-layer plates with the solvent system[14] was obtained from Toyo Kagaku Sangyo (Tokyo, Japan). The UV light sources (253.6 and 365.0 nm) were supplied by Manasuru Kagaku Kogyo (Tokyo, Japan).

Solvent systems for thin-layer chromatography

The following solvent systems were used: (1) *n*-hexane, (2) benzene, (3) ethyl acetate, (4) acetone, (5) acetonitrile, (6) methanol and (7) *n*-hexane–ethyl acetate–methanol–formic acid.

Application of samples and development of chromatographic plates

Samples of volume 0.5–1.0 μl of each test solution were spotted with a capillary on to a starting line 2 cm from the lower edge of the plates. The chamber containing the solvent system was partially lined with filter paper soaked in the solvent system.

The plate was placed in the chamber and equilibrated with the solvent vapour for 20 min (for solvents 1–6) or 1 h (for solvent 7) before commencing development. The development was carried out at 20 ± 1°C by immersing the plate in the solvent system to a depth of *ca.* 0.5 cm without opening the cover of the chamber, and was continued until the solvent front had travelled 10 cm from the starting line.

Detection of spots on the plates

Quinoline, isoquinoline and their methyl derivatives were observed under UV light (253.6 nm) as dark spots, while the benzoquinolines, 10-azabenzo[*a*]pyrene and the dibenzacridines were observed under UV light (365.0 nm) as fluorescent spots.

RESULTS AND DISCUSSION

Development with single solvents

The R_F values of the azaarenes obtained using various single solvents for development are listed in Table II. When solvents more polar than *n*-hexane were employed, the spots of all the azaarenes except 10-azabenzo[*a*]pyrene and the dibenzacridines showed considerably less tailing. When benzene was used for development only the separation of 10-azabenzo[*a*]pyrene and the dibenzacridines from the others was successful. When developed with acetone, acetonitrile or methanol, the larger azaarene molecules were distributed at lower R_F values, showing a pattern typical of

TABLE II

R_F VALUES OF AZAARENES

The number of asterisks represents the degree of tailing of the spot.

Compound No.	*Solvent system*					
	n-Hexane	*Benzene*	*Ethyl acetate*	*Acetone*	*Acetonitrile*	*Methanol*
1	0.57***	0.70	0.69	0.74	0.68	0.71
2	0.45***	0.65	0.68	0.73	0.65	0.69
3	0.62***	0.72	0.72	0.75	0.67	0.71
4	0.61***	0.67	0.68	0.74	0.64	0.69
5	0.59***	0.68	0.69	0.74	0.64	0.69
6	0.62***	0.68	0.69	0.74	0.63	0.69
7	0.82***	0.74	0.73	0.75	0.64	0.65
8	0.57***	0.68	0.70	0.73	0.66	0.69
9	0.72***	0.70	0.70	0.74	0.62	0.69
10	0.68***	0.72	0.71	0.74	0.63	0.69
11	0.67***	0.72	0.71	0.75	0.64	0.69
12	0.39***	0.66**	0.63**	0.65*	0.50**	0.55*
13	0.32***	0.62*	0.62	0.66	0.49	0.54
14	0.56***	0.71	0.67*	0.68	0.53*	0.48
15	0.37***	0.64*	0.63*	0.67	0.50**	0.54*
16	0.48***	0.70*	0.69	0.71	0.63*	0.63
17	0.00	0.42***	0.34***	0.26***	0.13***	0.06*
18	0.00	0.51***	0.27***	0.24***	0.10***	0.03*
19	0.00	0.57***	0.36***	0.29***	0.12***	0.02*
20	0.00	0.45***	0.30***	0.24***	0.12***	0.03*

reversed-phase partition chromatography. This agreed with the results previously obtained on polyamide by development with acetone–water (6:4) and methanol–water (8:2)[5]. However, derivatives and isomers of azaarenes containing the same number of aromatic rings were separated from each other hardly at all.

Development with n-hexane–ethyl acetate–methanol–formic acid systems.

In order to determine which solvent systems are most suitable for the separation of isomers and derivatives of azaarenes containing the same number of aromatic rings, development was carried out with a series of solvent systems composed of *n*-hexane–ethyl acetate–methanol–formic acid (100 − *A*:*A*:5:5; *A* varied from 30 to 50).

When developed with *n*-hexane–ethyl acetate–methanol–formic acid (60:40:5:5), the azaarenes tested were distributed as shown in Fig. 1. Quinoline, isoquinoline and their monomethyl derivatives were distributed, with a good separation, between R_F 0.26 and 0.77. The distribution was wider than those obtained using single solvent systems, or those with acetone–water or methanol–water systems[5]. Such a good separation has not been obtained using TLC with other adsorbents.

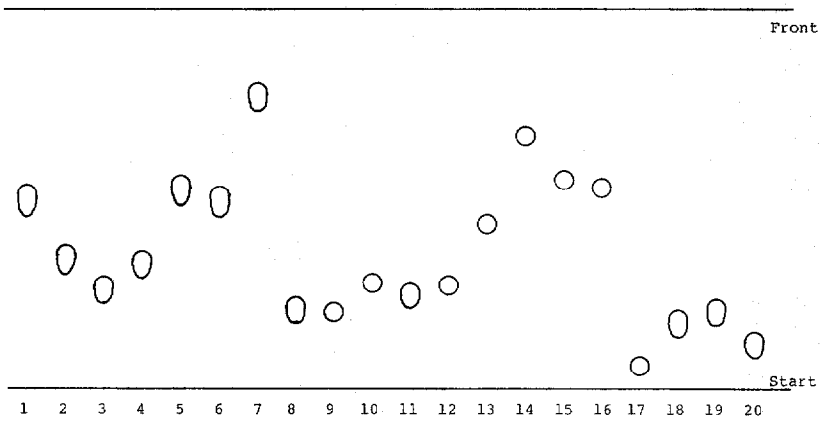


Fig. 1. Chromatogram of azaarenes on polyamide layers. Solvent systems: *n*-hexane–ethyl acetate–methanol–formic acid (60:40:5:5). Numbers of azaarenes correspond to those shown in Table I.

It is known that the effect of the position of the methyl substituent in the quinoline molecule is related to the interaction of the nitrogen atom of the molecule with the polyamide used. The R_F values of the methyl derivatives of quinoline decreased in the order of substitution 8-, 6-, 7-, 4-, 2-methyl.

As the basicity of the nitrogen atom in the molecules depends on the (+) inductive effect of the methyl group, 2-methylquinoline gives the greatest inductive effect on the lone pair of the nitrogen atom and shows the lowest R_F value amongst the monomethyl derivatives. The basicity of compounds in which the position of the methyl substituent is farther from the nitrogen atom decreases as the distance between them increases; these compounds showed higher R_F values. On the other hand, the 8-methyl derivative has the weakest basicity among the monomethyl derivatives of

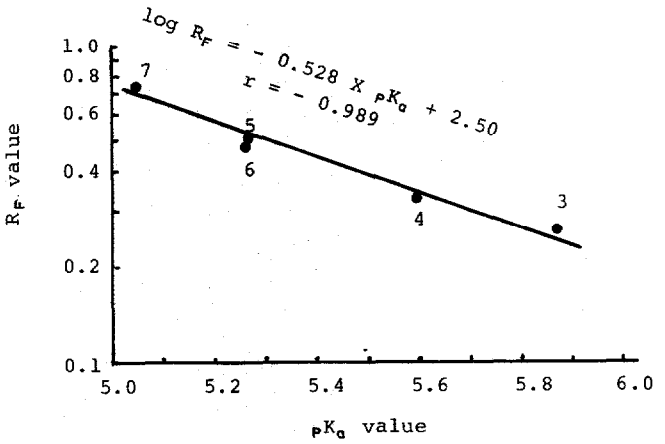


Fig. 2. Relationship between the pK_a values of the monomethylquinolines and their R_F values. Solvent system: *n*-hexane–ethyl acetate–methanol–formic acid (60:40:5:5). Numbers of azaarenes correspond to those shown in Table I.

quinoline because the lone pair of the nitrogen atom interacts with the methyl group in the flat molecule, inducing the highest R_F value.

1-Methylisoquinoline showed a lower R_F value than isoquinoline. This is due to the methyl group having a similar effect as in the qinoline derivatives.

The relationships between the available pK_a values for the monomethyl quinolines[15] and their log R_F values obtained by development with *n*-hexane–ethyl ac-

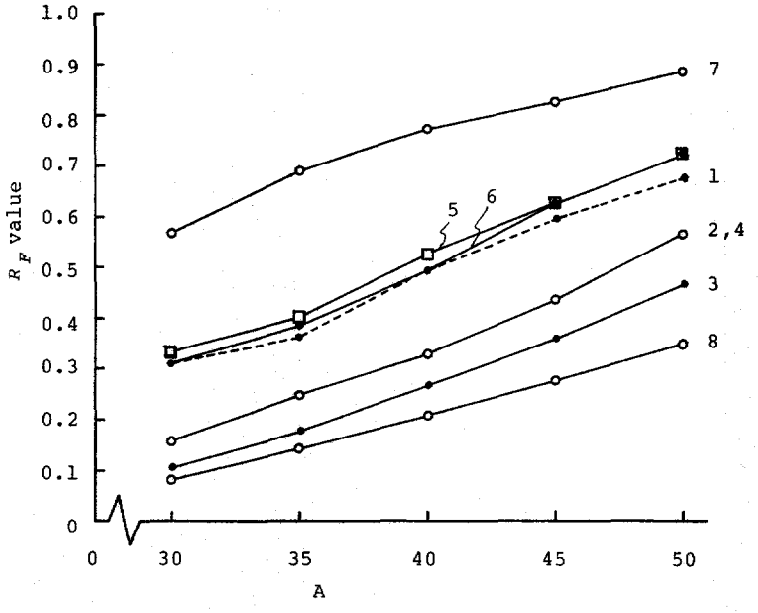


Fig. 3. Relationship between the distribution of quinoline, isoquinoline and their monomethyl derivatives and the proportion of ethyl acetate (*A*) in the solvent system. Solvent system: *n*-hexane–ethyl acetate–methanol–formic acid (100 − *A*:*A*:4:4). Numbers of azaarenes correspond to those shown in Table I.

etate–methanol–formic acid (60:40:5:5) is shown in Fig. 2. A strong correlation is observed.

As is shown in Fig. 3, the proportions of *n*-hexane and ethyl acetate in the solvent systems dit not have much effect on the separation of quinoline and the monomethyl derivatives, although their R_F values became relatively high as the amount of ethyl acetate in the system increased.

Dimethyl derivatives in which one of the methyl groups is commonly substituted in the 2-position (2,4-, 2,6- and 2,7-dimethylquinoline) gave a distribution which had R_F values lower than those of the monomethyl derivatives of quinoline. When developed with solvent systems in which the ratios of ethyl acetate to *n*-hexane were higher, these compounds showed a better separation, as shown in Fig. 4. A better separation might be obtained by using solvent systems in which *A* is greater than 50.

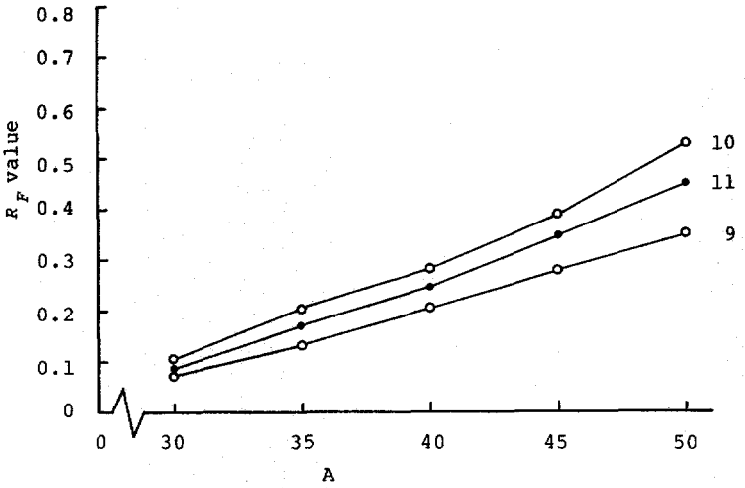


Fig. 4. Relationship between the distribution of the dimethylquinolines and the proportion of ethyl acetate (*A*) in the solvent system. Solvent system: *n*-hexane–ethyl acetate–methanol–formic acid (100 − *A*:*A*:5:5). Numbers of azaarenes correspond to those shown in Table I.

As the inductive effect of a methyl substituent in the 2-position of the molecule on the lone pair of the nitrogen atom is greater than that of any other methyl group, the dimethyl derivatives were distributed near to 2-methylquinoline. However, the relative distribution of the dimethyl derivatives is similar to that of the 4-, 6- and 7-methyl derivatives of quinoline.

When the benzoquinolines were developed using the *n*-hexane–ethyl acetate–methanol–formic acid systems, the compounds were separated completely with large differences in their R_F values. These decreased in the order benzo[*h*]quinoline, phenanthridine, benzo[*f*]quinoline, acridine, as shown in Fig. 5. The separation became poorer when solvent systems in which *A* was greater than 45 were used. The separation of azaarenes containing three rings was superior to that obtained not only by TLC on silica gel[9] and alumina[6,8] but also by paper partition chromatography[4,5].

When *n*-hexane–ethyl acetate–methanol–formic acid (60:40:5:5) was used for

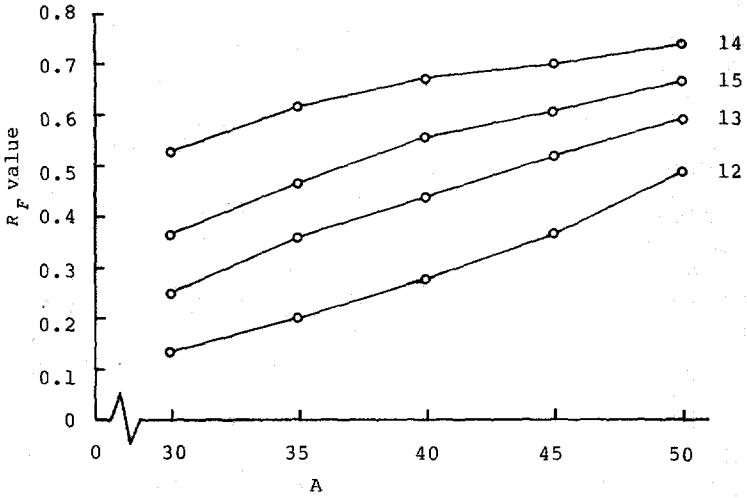


Fig. 5. Relationship between the distribution of the benzoquinolines and the proportion of ethyl acetate (A) in the solvent systems. Solvent system: *n*-hexane–ethyl acetate–methanol–formic acid (100 − A:A:5:5). Numbers of azaarenes correspond to those shown in Table I.

development, benzo[*h*]quinoline (7,8-substitution), benzo[*f*]quinoline (5,6-substitution) and acridine (2,3-substitution) were distributed nearly at the same positions as the 8-, 6- and 2-monomethylquinolines, respectively. This is attributed to the inductive effect of the substituted aromatic ring on the stability of the lone pair of the nitrogen atom in the molecule as well as that of the methyl substituent.

Fig. 6 shows that the available pK_a values of the azaarenes containing three rings[16] are closely correlated to their R_F values obtained by development with the abovementioned solvent system. When the pK_a values of isomers of the three-ring azaarenes are known, their R_F values may be assumed from the correlation.

Separation of the three dibenzacridines and 10-azabenzo[*a*]pyrene using the *n*-hexane–ethyl acetate–methanol–formic acid systems was poorer than when silica gel plates were used with pentane–diethyl ether (97:3)[9] as solvent. The proportions of ethyl acetate in the systems did not have any effect on the separation. The R_F values decreased very slightly in the order dibenz[*c*,*h*]acridine, dibenz[*a*,*h*]acridien, 10-azabenzo[*a*]pyrene, dibenz[*a*,*j*]acridine. This order is related to the basicity of the nitrogen atom of their molecules as follows. The lone pair of the nitrogen atom in the dibenz(*c*,*h*]acridine molecule has an affinity to the protons of both the aromatic rings at the *c*- and *h*-positions of acridine, and this compound shows the weakest basicity among the five-ring azaarenes. On the other hand, dibenz[*a*,*j*]acridine, in which the lone pair of the nitrogen atom is not affected by the aromatic rings at the *a*- and *j*-positions, has the strongest basicity among the five-ring azaarenes, and shows the lowest R_F value. The chromatograms in which the five-ring azaarenes were poorly separated and in which dibenz[*c*,*h*]acridine showed a lower R_F value than acridine demonstrate that the distribution of the five-ring azaarenes is more closely related to molecular size than to the basicity of the nitrogen atom.

Although the azaarenes consisting of two and three aromatic rings were widely distributed on the plates by the systems employed, azaarenes with similar basicities

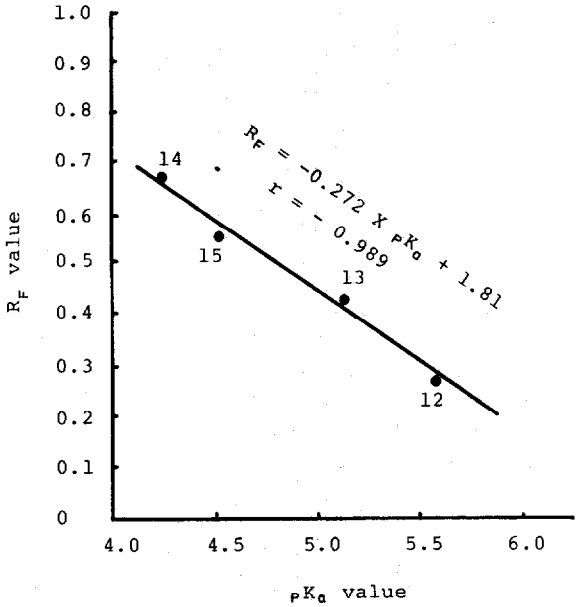


Fig. 6. Relationship between the pK_a values of the benzoquinolines and their R_F values. Solvent system: *n*-hexane–ethyl acetate–methanol–formic acid (60:40:5:5). Numbers of azaarenes correspond to those shown in Table I.

were not separated. In the detection of azaarenes, therefore, it is necessary first to fractionate the azaarenes into groups having the same number of aromatic rings by development with single solvents such as methanol, and subsequently to separate the azaarenes in each group by development with an *n*-hexane–ethyl acetate–methanol–formic acid system. Such a combination of TLC systems using polyamide should make the detection of azaarenes easy and reliable. Furthermore, the pre-separation of azaarenes by TLC using two different solvent systems should prove very useful to qualitative and quantitative HPLC and GLC analysis.

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