CHROM. 3627

Paper chromatography of aza-heterocyclic hydrocarbons

III. Some further paper and thin layer systems

In two recent communications from this laboratory we have examined a series of paper chromatographic systems for the separation of aza-heterocyclic hydrocarbons[1,2]. As mixtures encountered in air pollution studies involve possibly hundreds of compounds numerous chromatographic methods have been employed and still others will be needed.

TABLE I

R_F VALUES OF AZA-HETEROCYCLICS ON PAPER DEVELOPED WITH AQUEOUS H_2SO_4

Paper: Whatman 3MM. Temperature: 18–20°. Eluant: aqueous H_2SO_4.

Substance	*0.1 N*	*0.5 N*	*1.0 N*	*2.0 N*	*3.0 N*
Acridine	0.77	0.74	0.73	0.71	0.71
2-Methylacridine	0.70	0.64	0.63	0.63	0.61
3-Methylacridine	0.70	0.64	0.64	0.62	0.61
Benz(*a*)acridine	0.25	0.23	0.21	0.18	0.17
Benz(*c*)acridine	0.35	0.32	0.26	0.26	0.25
8,12-Dimethylbenz(*a*)acridine	0.21	0.18	0.17	0.14	0.13
7,9-Dimethylbenz(*c*)acridine	0.10	0.09	0.12	0.05	0.05
8,10-Dimethylbenz(*a*)acridine	0.14	0.12	0.10	0.06	0.04
7,10-Dimethylbenz(*c*)acridine	0.11	0.09	0.10	0.08	0.05
8,10-Dimethylbenz(*c*)acridine	0.13	0.11	0.09	0.07	0.04
9,12-Dimethylbenz(*a*)acridine	0.13	0.10	0.12	0.09	0.09
Dibenz(*a,h*)acridine	0.01	0.01	0.00	0.00	0.00
Dibenz(*a,j*)acridine	0.00	0.01	0.00	0.00	0.00
Pyrenoline	0.06	0.04	0.04	0.03	0.02
Benzo(*c*)cinnoline	0.48	0.64	0.69	0.78	0.77
9-(2′-Pyridyl)-anthracene	0.30	0.27	0.25	0.20	0.17
9-(3′-Pyridyl)-anthracene	0.18	0.15	0.15	0.12	0.10
9-(4′-Pyridyl)-anthracene	0.16	0.12	0.13	0.09	0.07
10-(2′-Pyridyl)-1,2-benzanthracene	0.02	0.01	0.00	0.01	0.01
10-(3′-Pyridyl)-1,2-benzanthracene	0.00	0.00	0.00	0.00	0.00
10-(4′-Pyridyl)-1,2-benzanthracene	0.00	0.00	0.00	0.00	0.00
14-Phenyldibenz(*a,j*)acridine	0.00	0.00	0.00	0.00	0.00
7-Phenyldibenz(*c,h*)acridine	0.00	0.00	0.00	0.00	0.00
Acridone	0.11				0.17
Phenanthridine	0.69	0.70	0.66	0.65	0.65
1-Azapyrene	0.47	0.43	0.42	0.41	0.35
4-Azapyrene	0.48	0.43	0.42	0.39	0.36
Benzo(*h*)quinoline	0.74	0.70	0.71	0.70	0.68
Benzo(*f*)quinoline	0.70	0.65	0.64	0.64	0.61
3-Methylbenzo(*f*)quinoline	0.71	0.65	0.62	0.58	0.50
7-Azafluoranthene	0.50	0.45	0.41	0.36	0.38
1-Azafluoranthene	0.60	0.54	0.49	0.47	0.46
1-Azacarbazol					
Acenaphtho(1,2*b*)acridine	0.48	0.44	0.42	0.37	0.36
Phenazine			0.70		0.81
Dibenzo(*a,c*)phenazine	0.00	0.00			0.00
11,12-Dimethyldibenz(*a,c*)phenazine					0.00

TABLE II

R_F VALUES OF AZO-HETEROCYCLICS DEVELOPED WITH ORGANIC ACIDS

Paper: Whatman 3MM. Temperature: 18–20°. Eluants: aqueous organic acids.

Substance	*CH_2Cl-COOH* 0.17 *N*	*$CHCl_2$-COOH* 0.17 *N*	*CCl_3-COOH* 0.17 *N*
Acridine	0.73	0.70	0.68
2-Methylacridine	0.61	0.63	0.58
3-Methylacridine	0.62	0.63	0.59
Benz(*a*)acridine	0.21	0.21	C
Benz(*c*)acridine	0.31	0.29	0.23
8,12-Dimethylbenz(*a*)acridine	0.18	0.20	0.00
7,9-Dimethylbenz(*c*)acridine	0.14	0.11	0.00
8,10-Dimethylbenz(*a*)acridine	0.15	0.10	0.00
7,10-Dimethylbenz(*c*)acridine	0.15	0.11	0.00
8,10-Dimethylbenz(*c*)acridine	0.07	0.09	0.00
9,12-Dimethylbenz(*a*)acridine	0.11	0.12	0.00
Dibenz(*a,h*)acridine	0.00	0.02	0.00
Dibenz(*a,j*)acridine	0.03	0.03	0.00
Pyrenoline	0.06	0.03	0.00
Benzo(*c*)cinnoline	0.34	0.53	C
9-(2′-Pyridyl)-anthracene	0.30	0.26	0.24
9-(3′-Pyridyl)-anthracene	0.16	0.15	0.08
9-(4′-Pyridyl)-anthracene	0.13	0.12	0.08
10-(2′-Pyridyl)-1,2-benzanthracene	0.02	0.03	0.00
10-(3′-Pyridyl)-1,2-benzanthracene	0.00	0.00	0.00
10-(4′-Pyridyl)-1,2-benzanthracene	0.00	0.00	0.00
14-Phenyldibenz(*a,j*)acridine	0.00	0.00	0.00
7-Phenyldibenz(*c,h*)acridine	0.00	0.00	0.00
Acridone	0.11	0.10	0.11
Phenanthridine	0.66	0.63	0.59
1-Azapyrene	0.38	0.38	C
4-Azapyrene	0.39	0.40	C
Benzo(*h*)quinoline	0.68	0.67	0.63
Benzo(*f*)quinoline	0.61	0.63	0.59
3-Methylbenzo(*f*)quinoline	0.62	0.62	C
7-Azafluoranthene	0.43	0.41	C
1-Azafluoranthene	0.50	0.46	0.00
1-Azacarbazol			C
Acenaphtho(1,2*b*)acridine	0.43	0.40	C
Phenazine	0.38	0.41	0.51
Dibenzo(*a,c*)phenazine	0.00	0.00	0.00
11,12-Dimethyldibenz(*a,c*)phenazine	0.00	0.00	0.00

Cl_3-COOH .5 N	COOH | COOH 0.1 N	COOH | COOH 0.5 N	COOH | CH_2 | COOH 0.1 N	COOH | $(CH_2)_2$ | COOH 0.1 N	COOH | CH_2 | COOH 0.5 N	CH_3-COOH 0.17 N (= 1%)
.74	0.72	0.78	0.68	0.58	0.77	0.54
.69	0.65	0.73	0.59	0.50	0.69	
.70	0.64	0.71	0.59	0.47	0.67	
.31	0.24	0.34	0.20	0.15	0.27	0.12
.33	0.33	0.42	0.26	0.16	0.36	0.10
.21	0.20	0.34	0.20	0.19	0.28	0.06
.00	0.11	0.22	0.10	0.07	0.15	0.07
.00	0.13	0.22	0.10	0.08	0.17	0.07
.13	0.13	0.23	0.10	0.07	0.18	0.06
.12	0.11	0.22	0.10	0.07	0.16	0.07
.16	0.11	0.25	0.10	0.09	0.18	0.00
.01	0.03	0.01	0.01	0.08	0.01	0.02
.00	0.02	0.03	0.02	0.02	0.03	0.01
.07	0.06	0.10	0.03	0.02	0.07	0.01
.71	0.48	0.69	0.32	0.30	0.48	0.29
.00	0.28	0.40	0.26	0.24	0.32	0.18
.00	0.16	0.28		0.00		0.00
.00	0.14	0.18	0.13	0.08	0.20	0.06
.03	0.03	0.05	0.27	0.02	0.05	0.02
.00	0.01	0.03	0.00			0.00
.00	0.01	0.02	0.01	0.00	0.02	0.00
.00	0.01	0.00	0.00	0.00	0.03	0.00
.00	0.00	0.00	0.00	0.00	0.00	0.00
.17	0.13	0.19	0.09	0.10	0.09	0.22
.59	0.65	0.72	0.64	0.52	0.68	
.46	0.40	0.52	0.35	0.26	0.47	
.48	0.40	0.50	0.36	0.27	0.49	
.72	0.68	0.72	0.65	0.54	0.72	
.69	0.63	0.68	0.59	0.50	0.67	
.52	0.63	0.71	0.63	0.52	0.69	
.00	0.43	0.53	0.38	0.27	0.51	
.00	0.53	0.64	0.43	0.31	0.60	
	0.46	0.53		0.31		0.29
	0.38	C		0.22		0.21
.00	0.00	0.00	0.00		0.00	0.00
.00	0.00	0.00	0.00	0.00	0.00	0.00

J. Chromatog., 37 (1968) 333–340

TABLE III

R_F VALUES OF AZA-HETEROCYCLICS ON PAPER DEVELOPED WITH ACETATE BUFFERS

Paper: Whatman 3MM. Temperature: 18–20°. Eluants: aqueous acetate buffers.

Substance	*pH*										
	0.48	*0.89*	*1.60*	*2.84*	*3.95*	*4.92*	*5.20*	*5.23*	*5.37*	*5.57*	*5.89*
Acridine	0.67	0.68	0.67	0.66	0.61	0.47	0.40	0.30	0.31	0.25	0.20
2-Methylacridine	0.57	0.60	0.59	0.57	0.51	0.31	0.25				
3-Methylacridine	0.58	0.60	0.58	0.58	0.52	0.30	0.25				
Benz(*a*)acridine	0.13	0.16	0.15	0.14	0.09	0.03	0.01				
Benz(*c*)acridine	0.22	0.25	0.25	C	0.00	0.00	0.00				
8,12-Dimethylbenz(*a*)acridine	0.13	0.15	0.15	0.14	0.09	0.03	0.00				
7,9-Dimethylbenz(*c*)acridine	0.06	0.07	0.05	0.05	0.03	0.00	0.00				
8,10-Dimethylbenz(*a*)acridine	0.07	0.09	0.08	0.06	0.02	0.00	0.00				
7,10-Dimethylbenz(*c*)acridine	0.07	0.09	0.08	0.07	0.03	0.00	0.00				
8,10-Dimethylbenz(*c*)acridine	0.07	0.08	0.09	0.06	0.03	0.00	0.00				
9,12-Dimethylbenz(*a*)acridine	0.06	0.07	0.09	0.19	0.05	0.03	0.02				
Dibenz(*a,h*)acridine	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Dibenz(*a,j*)acridine	0.00	0.00	0.00	0.00	00.0	0.00	0.00				
Pyrenoline	0.01	0.02	0.02	0.00	0.00	0.00	0.00				
Benzo(*c*)cinnoline	0.68	0.57	0.28	0.28	0.20	0.19	0.20	0.18	0.20	0.12	0.17
9-(2′-Pyridyl)-anthracene	0.22	0.20	0.24	0.20	0.14	0.08	0.08				
9-(3′-Pyridyl)-anthracene	0.13	0.12	0.07	0.00	0.00	0.00	0.00				
9-(4′-Pyridyl)-anthracene	0.07	0.07	0.10	0.05	0.00	0.00	0.00				

10-(2'-Pyridyl)-1,2-benzanthracene	0.00	0.00	0.00	00.0	0.00	0.00	0.00				
10-(3'-Pyridyl)-1,2-benzanthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
10-(4'-Pyridyl)-1,2-benzanthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
14-Phenyldibenz(*a,j*)acridine	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
7-Phenyldibenz(*c,h*)acridine	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Acridone	0.10	0.11	0.11	0.10	0.09	0.09	0.08				
Phenanthridine	0.60	0.61	0.65	0.56	0.37	0.12					
1-Azapyrene	0.33	0.35	0.36	0.27	0.10	0.03	0.02				
4-Azapyrene	0.33	0.37	0.35	0.24	0.10	0.03	0.02				
Benzo(*h*)quinoline	0.63	0.64	0.63	0.54	0.34	0.14	0.15				
Benzo(*f*)quinoline	0.56	0.58	0.58	0.56	0.46	0.21	0.17				
3-Methylbenzo(*f*)quinoline	0.55	0.57	0.56	0.59	0.53	0.33	0.29				
7-Azafluoranthene	0.36	0.36	0.36	0.26	0.10	0.03	0.04				
1-Azafluoranthene	0.42	0.44	0.42	0.24	0.09	0.02	0.02				
1-Azacarbazol	0.57	0.57	0.55	0.31	0.12	0.06	0.05				
Acenaphtho(1,2*b*)acridine	0.37	0.38	0.37	0.23	0.11	0.04	0.05				
Phenazine	0.64	0.48	0.28	0.20	0.21	0.19	0.17	0.12	0.12	0.11	0.12
Dibenzo(*a,c*)phenazine	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
11,12-Dimethyldibenz(*a,c*)phenazine	0.00	0.00	0.00	0.00	0.00	0.00	0.00				

pH 0.48 to pH 5.20: HCl/CH_3COONa buffers.
pH 5.23 to pH 5.89: CH_3COOH/CH_3COONa buffers.

In this note we report on results obtained with some further paper chromatographic systems, as well as with polyamide thin layers.

Paper chromatographic systems using aqueous acids as eluants

Preliminary results indicated that sulphuric acid gave some improved separations of some pairs of low R_F compounds.

Table I reports R_F values for various concentrations of aqueous sulphuric acid. There is a general trend of decreasing R_F values with the increase in the H_2SO_4 concentration, except for benzo-(*c*)-cinnoline.

Table II shows the R_F values for various concentrations of some organic acids. The chloracetic acids, with the exception of monochloracetic acid, precipitated many

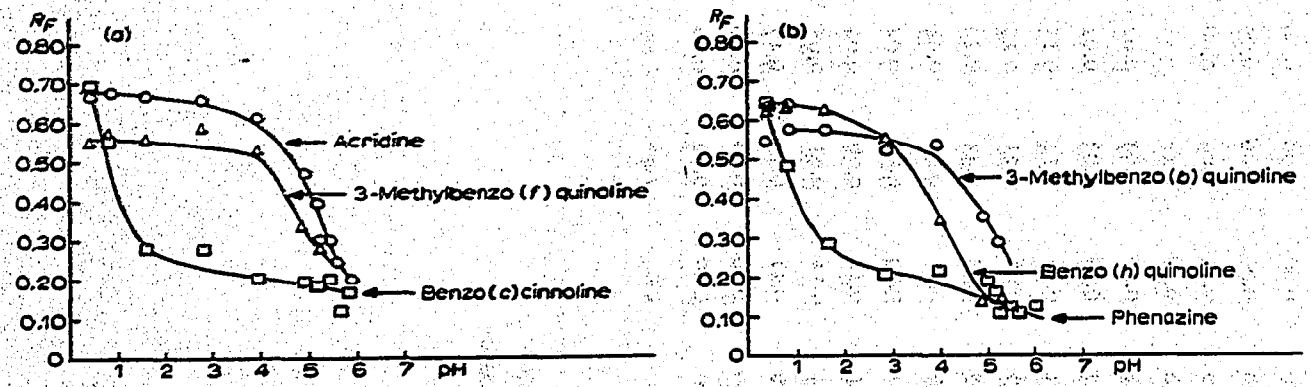

Fig. 1a and b. Some R_F–pH curves for aza-heterocyclics developed with acetate buffers on cellulose paper. Benzoquinolines and acridine have similar curves and can be distinguished clearly from phenazine or benzo(*c*)cinnoline.

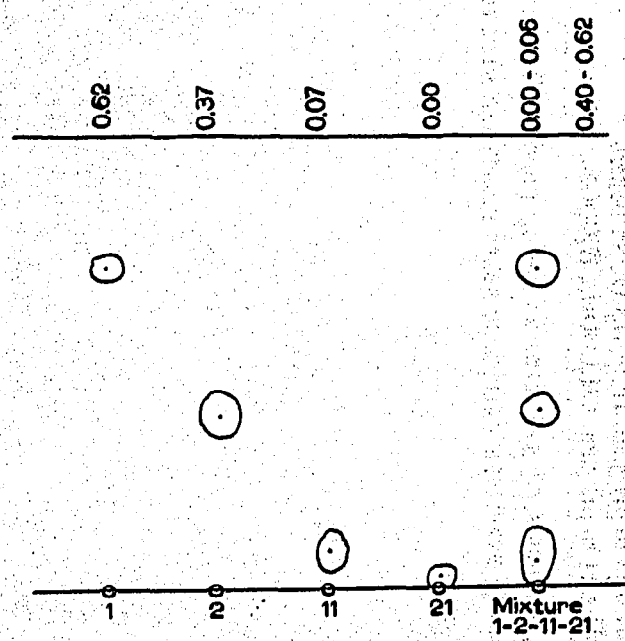

Fig. 2. Polyamide thin layer chromatogram. Solvent: methanol 80%, water 20%. Distance start to solvent front: 69 mm. 1 = Acridine; 2 = benz(*a*)acridine; 11 = dibenz(*a*,*j*)acridine; 21 = 7-phenyldibenz(*c*,*h*)acridine. Mixture 1, 2, 11 and 21.

acridines wholly or partially on the point of application and are hence of little use for analytical work. Dicarboxylic acids gave higher R_F values for many acridines than acetic acid; however the sequence remains essentially unchanged.

Paper chromatographic systems with aqueous buffers

There is a marked change of R_F value with all aza-heterocyclics when the pH values are changed by using aqueous acetate buffers as eluants. Table III and Fig. 1 show the possibilities as well as the limitations of pH variation, the latter being that no information can be drawn from low R_F values and that most monoaza-heterocyclics have an inflection point in the same pH range. Comparisons of pK values with paper

TABLE IV

R_F VALUES OF AZA-HETEROCYCLICS ON POLYAMIDE THIN LAYERS

Polyamide thin layers (Cheng Chin Trading Co. Ltd., Taiwan). Temperature: 18–20°.

Substance	*Acetone–water 60%–40%*	*Methanol–water 80%–20%*
Acridine	0.62	0.63
2-Methylacridine	0.58	0.59
3-Methylacridine	0.64	0.62
Benz(*a*)acridine	0.30	0.28
Benz(*c*)acridine	0.23	0.15
8,12-Dimethylbenz(*a*)acridine	0.28	0.16
7,9-Dimethylbenz(*c*)acridine	0.13	0.08
8,10-Dimethylbenz(*a*)acridine	0.11	C
7,10-Dimethylbenz(*c*)acridine	0.16	0.12
8,10-Dimethylbenz(*c*)acridine	0.12	C
9,12-Dimethylbenz(*a*)acridine	0.32	0.46
Dibenz(*a,h*)acridine	0.04	0.00
Dibenz(*a,j*)acridine	0.05	C
Pyrenoline	0.14	0.13
Benzo(*c*)cinnoline	0.73	
9-(2′-Pyridyl)-anthracene	0.37	0.48
9-(3′-Pyridyl)-anthracene	0.41	0.43
9-(4′-Pyridyl)-anthracene	0.40	0.45
10-(2′-Pyridyl)-1,2-benzanthracene	0.18	0.26
10-(3′-Pyridyl)-1,2-benzanthracene	0.21	0.25
10-(4′-Pyridyl)-1,2-benzanthracene	0.09	C
14-Phenyldibenz(*a,j*)acridine	0.00	C
7-Phenyldibenz(*c,h*)acridine	0.00	0.00
Acridone	0.41	0.41
Phenanthridine	0.66	0.62
1-Azapyrene	C	0.51
4-Azapyrene	C	0.45
Benzo(*h*)quinoline	0.65	0.60
Benzo(*f*)quinoline	0.63	0.60
3-Methylbenzo(*f*)quinoline	0.73	0.63
7-Azafluoranthene	0.51	0.51
1-Azafluoranthene	0.55	0.54
1-Azacarbazol	0.58	0.56
Acenaphtho(1,2*b*)acridine	0.55	0.51
Phenazine	0.72	0.70
Dibenzo(*a,c*)phenazine	0.00	0.00
11,12-Dimethyldibenz(*a,c*)phenazine	0.00	0.00

chromatographic behaviour could not be made because very few pK values were found in the literature.

The pH curves may be useful for deciding whether a certain unknown substance is of the acridine type or not.

Thin layer chromatography on ready polyamide layers

Polyamide layers have been used for many classes of compounds notably for phenols (for a review see HÖRHAMMER *et al.*[4]), however acridines have not been studied to our knowledge. Table IV shows the R_F values obtained on ready-made polyamide layers (Cheng Chin Trading Co. Ltd., Taiwan) with acetone–water and methanol–water as solvents. The order of movement is mainly according to molecular weight and hence the sequences are essentially similar to those on cellulose paper. However very clear and fast separations of several artificial mixtures could be obtained readily (see Fig. 2).

This work was carried out as part of the work for a doctorate thesis by S. CAROLI. Some of the work on sulphuric acid solvents was done by G. ROCH.

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