IDENTIFICATION OF AROMATIC COMPOUNDS AS SIMPLE AZINES BY PAPER AND THIN-LAYER CHROMATOGRAPHY

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(Received November 17th, 1966)

INTRODUCTION

Carbonyls are found in many types of mixtures. Because many carbonyls are volatile, they may not be detected when present in minute amounts. Hence, various types of derivatives have been used to identify them. Although the 2,4-dinitrophenylhydrazones have been most widely used, other derivatives may also be used. BRANDT *et al.*[6–7] prepared azines with the use of 2-diphenylacetyl-1,3-indandione 1-hydrazone, and chromatographed them on vaseline-treated paper. Recently, KLEMM *et al.*[14] chromatographed some azines on alumina.

In the work described here azines were produced by reacting the aromatic carbonyls with hydrazine or hydrazine dihydrochloride. The azines produced were chromatographed on paper and on silica gel in various systems.

EXPERIMENTAL*

Apparatus

Spectrophotometric measurements were made on a Cary Model 14, manufactured by Applied Physics Corporation.

Standard chromatographic equipment including plates, applicator of fixed thickness, and development tanks were obtained from Brinkman Instruments, Inc. The papers and plates were examined in a chromatovue cabinet, which is equipped with 3660 Å, 2537 Å and white light (Kensington Scientific Corporation).

Phosphorescence was checked with an Aminco-Keirs spectrophosphorimeter.

Reagents

Chemicals were reagent grade except where otherwise noted. Hydrazine derivatives were prepared by the method of SHAPIRO[19], LOCK AND STACH[15], or SZMANT AND MCGINNIS[23, 24].

Schleicher and Schüll paper 2040 a gl. was used for the paper chromatograms. Silica gel according to Stahl manufactured by E. Merck, Darmstadt, Germany, was used for the thin-layer chromatography.

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* Mention of commercial products does not constitute endorsement by the Public Health Service.

Paper chromatography

Azines were separated in the following systems: (A) papers were impregnated with 75 % N,N-dimethylformamide–25 % acetone using N,N-dimethylformamide–cyclohexane in the ratio of 20:80 v/v; (B) papers were impregnated with 50 % N,N-dimethylformamide–50 % acetone using N,N-dimethylformamide–cyclohexane in the ratio of 20:80 v/v as developing solvent; (C) papers were impregnated with 50 % N,N-dimethylformamide–50 % acetone using decalin–N,N-dimethylformamide in the ratio 80:20 v/v; (D) papers were impregnated with 75 % N,N-dimethylformamide–25 % acetone using cyclohexane–N,N-dimethylformamide–ethyl acetate–water in the ratio 120:120:20:40 v/v[21]; (E) papers were impregnated with 75 % N,N-dimethylformamide–25 % acetone using SCHNITTS[18] system of dibutyl ether–N,N-dimethylformamide–tetrahydrofuran in the ratio 85:15:4 v/v; (F) papers were impregnated with 25 % N,N-dimethylformamide–75 % ethyl alcohol (95 %) using cyclohexane–N,N-dimethylformamide–carbon tetrachloride in the ratio 20.1:4 v/v[8]; and (G) papers were impregnated with 10 % methylformanilide–90 % ethyl alcohol (95 %) using cyclohexane–methylformanilide in the ratio 4:1 v/v as the developing solvent.

Determination of R_F values. Solutions of pure azines of approximately 1 mg per ml in ethyl alcohol (95 %) were chromatographed at 20° by the procedure of ascending chromatography[10, 11]. Most of the spots could be located by means of ultraviolet light. Others were located by spraying the chromatogram with one of the following reagents: equal volumes of an aqueous mixture of 10 % ferric chloride and 1 % of potassium ferricyanide, mixed a few minutes prior to use[2]; a 5 % aqueous solution of tetraethylammonium hydroxide[1]; or a 0.1 % solution of *p*-dimethylaminocinnamaldehyde in 95 % ethyl alcohol plus 1 % hydrochloric acid by volume. The results are presented in Table I.

Fluorescence and phosphorescence studies were performed according to SAWICKI *et al.*[16, 17]. Analyses were performed by the Galbraith Laboratories, Inc., Knoxville, Tenn.

Thin-layer chromatography

Chromatoplates 20 by 20 cm were prepared by the method described by STAHL[20], with silica gel as adsorbent. A layer 250 μ thick was used on all plates. The plates were activated at 110–120° for 1 h and stored in a desiccator until used. The chromatographic tanks were lined with filter paper and equilibrated from 4 to 5 h before the plates were chromatographed. The following systems were employed: (A) benzene; (B) ethyl acetate–hexane in the ratio 1:1 v/v; (C) benzene–paraffin–acetic acid–water in the ratio 55:20:45:30 v/v. The solvents were mixed in a separatory funnel and allowed to equilibrate from 30–40 min. The two phases were then separated. The benzene phase was placed on the bottom of the chromatographic tank, while the aqueous phase was placed in a small beaker inside the tank; thus the tank was equilibrated in respect to both phases; (D) tetrahydrofuran–dibutyl ether in the ratio 4:85, v/v; (E) dichloromethane; (F) decalin–dichloromethane–methyl alcohol in the ratio 5:4:1 v/v.

Determination of R_F values on thin-layer plates. A solution (10–15 μl) of azines of approximately 1 mg per ml was placed at intervals on the plate, 1.5 cm from the base of the coated plate. The plates were allowed to develop to a height of 10 to 11 cm. The plates were first examined in ultraviolet light, then sprayed with a mixture of ferric chloride and potassium ferricyanide. The results are recorded in Table IV.

RESULTS AND DISCUSSION

The results in Table I summarize the mean R_F values (12–15 runs) of the azines of aromatic carbonyls in (A) N,N-dimethylformamide–cyclohexane in comparison with the values obtained with the other systems as determined by the method of ascending chromatography at 20°. With system A, in the benzalazine series, azines with hydroxyl substituents display lower R_F values than benzalazine proper. A methoxy substituent likewise lowers the R_F value. Methoxy or hydroxy substituents in the *ortho* position have approximately the same effect on the R_F values. Thus, *o*-methoxybenzalazine and salicylaldazine cannot be separated in system A, but this pair can be separated in system B. When a benzalazine has both a methoxy and a hydroxy group attached, the combined effect is to produce an R_F value that is lower than the R_F

TABLE I

PAPER CHROMATOGRAPHIC PROPERTIES OF AZINES OF AROMATIC CARBONYLS

Paper: Schleicher and Schüll a gl.

Substance	$R_F \times 100$						
	A	*B*	*C*	*D*	*E*	*F*	*G*
Benzalazine	54	65	56	75		83	42
2-Hydroxybenzalazine	2	2	3	53	21	2	
Salicylaldazine	31	23	24	45		56	
2-Methoxybenzalazine	31	36	41	67	55	69	28
Anisaldazine	22	21	22	37		49	17
Vanillin azine	0			0	11	2	
4-Carboxybenzalazine	0						
2-Hydroxy-3-methoxybenzalazine	3		4	41	32	12	
2,3-Dimethoxybenzalazine	20			64	61	68	
4-Acetoaminobenzalazine	0			1	11	1	
4-Dimethylaminobenzalazine	8			22	44	79	
Veratraldazine	4			4	13	13	
3,4,5-Trimethoxybenzalazine	4			30	17	16	
Piperonal azine	9	9	8	41	37	32	
Acetophenone azine	62			80		92	52
2-Hydroxyacetophenone azine	33	44	38	64	59	68	
4-Hydroxyacetophenone azine	0			0	17	2	
2,5-Dihydroxyacetophenone azine	2			5	31	1	
2,4-Dihydroxyacetophenone azine				3	8		
2,6-Dihydroxyacetophenone azine				5	5		
4-Aminoacetophenone					4		
4-Aminoacetophenone azine hydrochloride	3			2			
2,4-Dimethylacetophenone azine	84		4	97			58
2,5-Dimethylacetophenone azine	86			96		95	67
3,4-Dimethylacetophenone azine	79			92		95	57
Propiophenone azine	78	84	75	91			60
Butyrophenone azine	86			92		98	73
Benzophenone azine	57	70	55	85	71	95	42
4-Hydroxybenzophenone azine	0			43	27	7	
2-Hydroxy-5-methylbenzophenone azine	41			94	70	93	
2-Hydroxy-4-methoxybenzophenone hydrazone	19					20 49	
2-Hydroxy-4-methoxybenzophenone azine	12			92	42	71	
2,4-Dihydroxybenzophenone azine					5		
Terephthaladazine	30				14 74		

value would have been had only a single substituent been attached. The specific value produced depends to a large extent on the position of the substituents. Carboxyl, acetyl, and methyl substituents on the benzalazine also result in lower R_F values.

Non-substituted ketazines show higher R_F values in system A than the aldazines. These R_F values increase from acetophenone to butyrophenone. The effects of hydroxy substituents on the ketazines are the same as on benzalazine. In the acetophenone series, methyl substituents increase the R_F value as expected. The position of the methyl substituent seems to have little effect upon the R_F value as displayed by 2,4-dimethylacetophenone azine, 2,5-dimethylacetophenone azine, and 3,4-dimethylacetophenone azine. The R_F value for butyrophenone azine (having the same number of carbon atoms) is about the same as that in the dimethylacetophenone azines in all systems except system G.

In the benzophenone series the substitution of hydroxyl groups on benzophenone results in lower R_F values. R_F values in general are higher in systems D and F than in system A, possibly because of the more polar nature of the solvents and possibly because of the percentage of the impregnating material.

Azines that show low R_F values in system A generally show higher R_F values in system E. System G was used to separate compounds with high mobilities in the other systems. Although lower R_F values are shown, separations are incomplete as shown by the dimethylacetophenone azines.

Although most of the azines studied can be detected under ultraviolet light as purple, yellow, or green spots, 2,4-dimethylacetophenone azine and 2,5-dimethylacetophenone azine are invisible by this method. They can be rendered visible blue by spraying with the potassium ferricyanide–ferric chloride solution, by using the *p*-dimethylaminocinnamaldehyde solution referred to earlier, or by spraying with a 5 % aqueous solution of tetraethylammonium hydroxide. The colors produced with *p*-dimethylaminocinnamaldehyde are pink to purple. Spraying with a 5 % aqueous solution of tetraethylammonium hydroxide results in yellow areas with the azines[1].

Table II gives data on melting points, analysis, and absorption for some of the compounds studied. Data for other compounds can be found in the literature[3–5,9,12–13,23,25–26].

Recently work has been done on characterizing compounds after paper and thin-layer chromatography by their fluorescence and phosphorescence colors both at room temperature and at liquid nitrogen temperature[16–17,22]. Table III gives data on the fluorescence and phosphorescence of some of the compounds studied. While very few of the compounds show phosphorescence, many show fluorescence at room temperature[26] and liquid nitrogen temperature.

The results in Table IV give a survey of mean R_F values (8–10 runs) on silica gel in: (A) benzene; (B) ethyl acetate–hexane; (C) benzene–paraffin–acetic acid–water; (D) dibutyl ether–tetrahydrofuran; (E) dichloromethane; and (F) decalin–dichloromethane–methanol. In system A, compounds having the *o*-hydroxy substituents seem to increase the R_F values, while hydroxyl substituents in other positions result in a decrease of R_F values. *o*-Methoxy and 2,3-dimethoxy benzalazines give two spots in benzene while *p*-dimethylaminobenzalazine and 2,5-dihydroxyacetophenone azine give multiple spots in system B. In system C, vanillin azine, and veratralaldehyde azine give two spots. In system F, *o*-methoxybenzalazine, vanillin azine, and veratraldazine give multiple spots. In system B, higher R_F values are shown for

TABLE II

PROPERTIES OF AZINES

Azine	*m.p. hot stage °C*	*Formula*	*Carbon*		*Hydrogen*		*Nitrogen*		*Oxygen*		*$\lambda_{max.}$*	*Log ε*	*Solvent*
			Calc.	*Found*	*Calc.*	*Found*	*Calc.*	*Found*	*Calc.*	*Found*			
2,5-Dimethylacetophenone azine	86–87	$C_{20}H_{24}N_2$	82.12	81.91	8.29	8.28	9.58	9.86	—	—	247	4.11	1,4-Dioxane
2,4-Dimethylacetophenone azine	84.5–85.5	$C_{20}H_{24}N_2$	82.12	81.91	8.29	8.31	9.58	9.72	—	—	251	4.30	EtOH
3,4-Dimethylacetophenone azine	118.5–120	$C_{20}H_{24}N_2$	82.12	82.08	8.29	8.24	9.58	9.66	—	—	275 214	4.47 4.51	EtOH
4-Carboxybenzalazine	347 turns brown. Decomp. 355	$C_{16}H_{12}N_2O_4$	64.84	64.65	4.10	4.27	9.46	9.24	21.61	21.75	313 325	4.48 4.43	EtOH
2-Hydroxy-5-methylbenzophenone azine	165–165.5	$C_{28}H_{24}N_2O_2$	79.95	79.72	5.77	5.72	6.66	6.50	7.61	7.80	388 306 250	4.20 4.36 4.08	EtOH
2,6-Dihydroxyacetophenone azine	Dec. 323–326	$C_{16}H_{16}N_2O_4$	63.97	63.70	5.38	5.35	9.33	9.42	21.32	21.24	333	4.28	EtOH
2,4-Dihydroxyacetophenone azine	Dec. 319–324	$C_{16}H_{16}N_2O_4$	63.97	60.25	5.38	5.58	9.33	8.73	21.32	24.80	275 305	4.50 4.13	1,4-Dioxane
3,4,5-Trimethoxybenzal azine	198–199	$C_{20}H_{24}N_2O_6$	61.82	61.83	6.23	6.26	7.21	7.30	24.73	24.58	335	4.60	EtOH
4-Hydroxybenzophenone hydrazone		$C_{13}H_{12}N_2O$	73.58	73.34	5.66	5.90	13.21	13.09	7.55	7.71			

TABLE III

FLUORESCENCE AND PHOSPHORESCENCE OF AZINES

Substance	*Room-temperature fluorescence*						*Low-temperature fluorescence*		
	Wet			*Dry*					
	Neutral	*Acid*	*Basic*	*Neutral*	*Acid*	*Basic*	*Neutral*	*Acid*	*Basic*
3-Hydroxybenzalazine	None	Pl Gr	Pl Yel Or	None	Pl Yel Gr	Pl Yel Or	None	Str Bl	Yel Gr
Salicylaldazine	Yel	None	Gr	Yel Gr	Yel Gr	Gr	Yel	—	Bl Gr
5-Nitrosalicylaldazine	Vr Pl Yel Gr	Vr Pl Yel Gr	Vr Pl Yel Gr	Vr Pl Yel Gr	Yel Gr	None	Yel Gr	Yel Gr	Yel Gr
2-Methoxybenzalazine	None	Pl Gr	None	None	Gr	None	Vr Pl Pk	Str Bl	None
Anisaldazine	None	Gr	None	None	Gr	None	None	Bl Gr	None
Vanillin azine	Yel Or	Pl Gr	Bl Gr	Yel Or	Pl Gr	Gr	Bl Gr	Bl Gr	Gr
2-Hydroxy-3-methoxybenzalazine	Rd Or	None	Yel Gr	Rd	Pl Rd	Gr	Rd Or	Gr	Gr
2,3-Dimethoxybenzalazine	None	Pl Yel Or	None	None	Yel Gr	None	None	Yel Gr	None
4-Acetoaminobenzalazine	None	Vr Pl Yel	Vr Pl Gr	None	Vr Pl Bl	Vr Pl Gr	None	Str Bl	Str Bl
3,4,5-Trimethoxybenzalazine	None	Yel Or	None	None	Yel Gr	None	None	Bl Gr	None
Veratraldazine	Vr Pl Gr	Yel	None	None	Yel	None	Bl Gr	Bl Gr	None
2-Hydroxyacetophenone azine	Yel	None	None	Gr	Gr	None	Gr	Bl	None
2,5-Dimethylacetophenone azine	None	None	None	None	Vr Pl Bl	None	Pl Bl	Str Bl	None
Terephthalaldazine	None	Bl Gr	None	None	None	None	None	Bl Gr	None

Bl = Blue; Gr = green; Vr = very; Or = orange; Rd = red; Str Bl = strong blue; Yel = yellow; Pl = pale; Pk = pink.

The following compounds gave negative results in the luminescence tests: (1) benzalazine; (2) acetophenone azine; and (3) propiophenone azine.

The following compounds gave negative results in the luminescence tests except in the following instances: (1) 4-Hydroxyacetophenone azine gave a green color under acid conditions at room temperature when dry; (2) at low temperature in acid solution piperonal gave a green color; (3) benzophenone gave a pale green color at low temperature under acid conditions.

All compounds were checked for phosphorescence with the instrument under neutral, acid and basic conditions. The results obtained were negative except in the following examples: with N,N-dimethylformamide as a solvent, 5-nitrosalicylaldazine and 4-acetoaminobenzalazine under acid conditions gave a very pale green phosphorescence. With chloroform as solvent piperonalazine gave a pale green phosphorescence under neutral conditions. With EPA (mixture of diethyl ether, isopentane and ethanol in ratio 5:5:2) as solvent, 4-hydroxyacetophenone azine gave a green phosphorescence under acid conditions, and 2,5-dimethylacetophenone azine gave a pale green phosphorescence under neutral conditions.

TABLE IV

THIN-LAYER CHROMATOGRAPHIC PROPERTIES OF AZINES OF AROMATIC CARBONYLS
Thin-layer: Silica Gel G.

Substance	$R_F \times 100$					
	A	*B*	*C*	*D*	*E*	*F*
Benzalazine	39	77	66	86		85
m-Hydroxybenzalazine	2	63	4	24	2	13
Salicylaldazine	59	78	72	87	85	81
2-Methoxybenzalazine	26 45	73	46		60	70 85
Anisaldazine	1	63	45	59		
Vanillin azine	2	24	15 30		18	17 38
2-Hydroxy-3-methoxybenzalazine	8	32[a]	30	34		61
2,3-Dimethoxybenzalazine	6 18	68		42	32	68
p-Dimethylaminobenzalazine	3	52 60			24	
p-Acetaminobenzalazine	2		0			
Veratralaldazine	2	43	21 32	16	24	54 66
Piperonal azine	15	76	54	64		
Acetophenone azine	35	67		89		70[a]
2-Hydroxyacetophenone azine	43	73	67	76	70	83
4-Hydroxyacetophenone azine	0	45		44	0	10
4-Aminoacetophenone azine	8	30				
2,4-Dihydroxyacetophenone azine	3	21[a]				
2,5-Dihydroxyacetophenone azine		34 46	11	16 32		
3,4-Dimethylacetophenone azine	3	91		93		
Propiophenone azine	48	84	72	93	84	69
Butyrophenone azine	63	89	74	94	91	74
Benzophenone azine	50	80	68	89	24	
4-Hydroxybenzophenone azine	0	75	5	49	12	
2,4-Dihydroxybenzophenone azine	3	39				
2-Hydroxy-4-methoxybenzophenone azine	28	76	53	63		
2-Hydroxy-4-methoxybenzophenone hydrazone			62 68 74			
2-Hydroxy-5-methylbenzophenone azine		37	70	81		

[a] Smears.

compounds that exhibit low R_F values in benzene. This is to be expected because of the polar nature of the solvent. The other systems were run for comparison. System C separates benzalazine and salicylaldazine by a wide margin, as expected.

Other adsorbents tried were cellulose, kieselguhr, and polyamide. The R_F values of most of the azines were identical on each adsorbent. On polyamide the fluorescences of the various compounds were increased or altered completely, but no extensive study was performed.

APPLICATIONS

An automobile exhaust sample collected in 1.0 N sodium hydroxide extracted with chloroform, and concentrated by a factor of 10, was examined qualitatively for the presence of carbonyls by thin-layer chromatography on silica gel in benzene. Salicylaldehyde was thought to be present. The sample was chromatographed on silica gel in benzene–paraffin–acetic acid–water for the purpose of distinguishing between salicylaldehyde and benzaldehyde. Salicylaldehyde was shown to be present each time. Some four other spots seen under ultraviolet light were observed. When the plate was sprayed with a 1% potassium ferricyanide–1% ferric chloride solution, blue spots appeared after the chromatoplate had dried indicating that a reducing substance was present.

A sample of diesel exhaust processed similarly was chromatographed on silica gel in benzene–acetic acid–paraffin–water. Some nine spots were revealed under ultraviolet light, but only one spot was positively identified as salicylaldehyde.

ACKNOWLEDGEMENTS

The author is indebted to HENRY JOHNSON for collecting the samples and to JACK PFAFF for performing the fluorescence and phosphorescence analyses.

SUMMARY

A method is described for the separation of carbonyls as simple azines by paper and thin-layer chromatography. Schleicher and Schüll 2040 a gl. papers were used in paper chromatography. Extensive data are given for separations on papers impregnated with N,N-dimethylformamide in: (1) N,N-dimethylformamide–cyclohexane; (2) dibutyl ether–tetrahydrofuran–N,N-dimethylformamide; (3) cyclohexane–N,N-dimethylformamide–carbon tetrachloride; (4) cyclohexane–N,N-dimethylformamide–ethyl acetate–water; and (5) decalin–N,N-dimethylformamide. Separations were also performed on papers impregnated with methylformanilide in cyclohexane–formanilide. Thin-layer separations were performed on silica gel in benzene; ethyl acetate–hexane; benzene–paraffin–acetic acid–water; dibutyl ether–tetrahydrofuran; dichloromethane; and decalin–dichloromethane–methanol.

An example of the thin-layer separation of an automobile exhaust sample is given.

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