IDENTIFICATION AND DETERMINATION OF UNUSUAL FUNCTIONAL GROUPS

E. SAWICKI

U.S. Department of Health, Education and Welfare, Cincinnati, Ohio 45226, U.S.A.

INTRODUCTION

Many of the unusual and unfamiliar analytical methods of yesterday have become the common methods of today. Therefore, when we speak of "unusual" functional groups we refer to those that are now novel but that may evolve with time and use into the usual and well-known.

With advances in knowledge of the physical and chemical properties of organic molecules, new types of functional groups are being found, some of which appear strange and unusual at the moment while others are more sharply delineated sub-divisions of well-known functional groups. The sub-divisional functional group contains the original functional group with additional atoms; for example, one sub-division of a ketonic C=O functional group is the $—CH_2—CO—CH_3$ grouping. Methods of analysis for compounds containing such a sub-divisional grouping are usually more highly selective.

Examples will be given of some well-known families of compounds, each theoretically containing a common functional group which, until recently, had not been exploited for analytical purposes.

Environmental health problems have proliferated tremendously in the past 40 years mainly because of the growing complexity of the chemical environment in which we live. Examples of this phenomenon are smog and the growing dinginess of the urban atmosphere; this dinginess indicates the accelerating infiltration of our air by man-made pollutants. Because of the increasing chemical pollution of our environment and its sometimes adverse effect on man, other life forms, and man-made objects, it is necessary first to determine the composition of the complicated mixtures with which we are in contact and then to decrease the concentrations of the harmful compounds. A combination of chromatographic and functional group methods has proved valuable in identifying and determining these chemicals. Functional group methods are being used in the direct analysis of many mixtures to detect or determine one compound or a family of compounds. Examples of a few types of unusual functional group methods will be given.

This paper will be concerned only with the unusual functional group methods that are useful in the picogram to microgram region.

AROMATIC HYDROCARBONS

A simple procedure for the estimation of aromatic compounds in complex mixtures is available[1, 2] and has been used in the estimation of benzo[*a*]-

pyrene in urban atmospheres[3]. The reagent is piperonal chloride, which forms a diarylmethane cationic dye, as shown in *Figure 1.* Under the conditions of the procedure the reagent attacks compounds more basic than benzene at their point of highest electron density, which must be an aromatic carbon with an attached hydrogen atom. This means that aromatic amines, aza heterocyclic compounds, nitroarenes, quinones, aromatic carbonyl compounds and polyhalo aromatic compounds do not react. Some of the compounds that do give positive results in the procedure are the methylated benzenes, the polynuclear aromatic hydrocarbons, phenols,

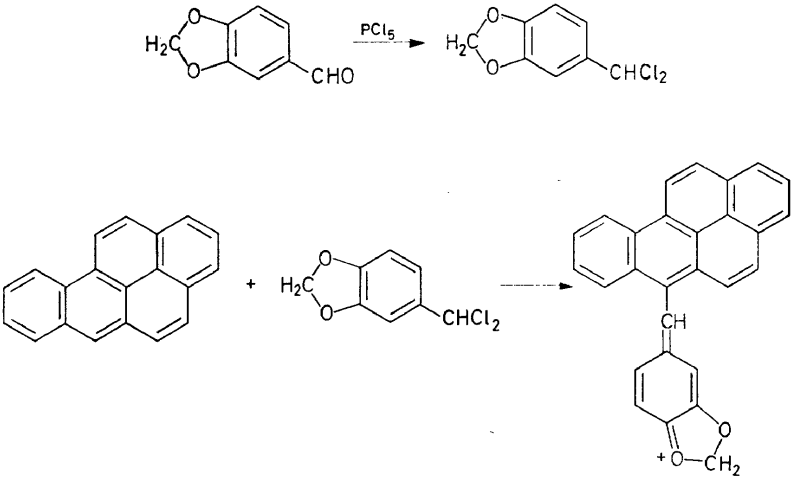


Figure 1. Equation for the determination of benzo[*a*]pyrene with piperonal

N-acylated aromatic amines[4], aromatic sulfides, aromatic ethers, and oxygen, sulfur, and imino heterocyclic compounds. The aromatic hydrocarbons that react with the reagent have a very wide range of basicity[5]; even hydrocarbons as strongly basic as azulene will react. However, on an alumina plate the piperonal test is much more selective[6]. Many polynuclear aromatic hydrocarbons that give positive results in solution give negative results on the plate.

Azulene and the polynuclear azulenes can be detected or determined by means of their spectral bands in the 500–700 mμ region if they are present in fairly large amounts. 4-Dimethylaminobenzaldehyde was the first of the electrophilic reagents found to give a hundred times better sensitivity than the direct method in the determination of the azulenes[7, 8]. Since then, nine more electrophilic reagents for the determination of the azulenes have been found[9]. These reagents should be capable of detecting or determining azulene derivatives and polynuclear aromatic hydrocarbon ring systems containing an azulene ring with the 1 or 3 position unsubstituted. The reaction of one of the reagents with azulene is shown in *Figure 2.*

Only the *p*-dimethylaminobenzaldehyde reagent has been used to locate and identify azulenes on paper chromatograms[10]. This functional group is

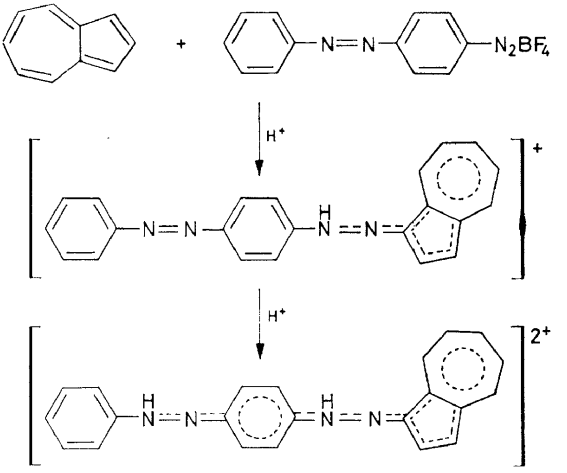
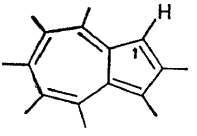


Figure 2. Equation for the determination of azulene with 4-phenylazobenzenediazonium fluoborate[9]

depicted by (I), which shows the 1 position as the position at which coupling takes place, especially if the 3 position is blocked.



(I)

Heterocyclic compounds containing structures iso-π-electronic to (I), where the CH=CH group in the seven-membered ring is replaced by an O, S, or NR group, would also be expected to react with the electrophilic reagents.

The cyclopentadienic CH_2 grouping is another functional group for which methods of analysis are available. The reaction of 1,2-dinitrobenzene, 1,4-dinitrobenzene, and 1,4-dinitronaphthalene with compounds containing the cyclopentadiene CH_2 group, in alkaline solution, results in a stable blue to green colour with long wavelength maxima ranging from 570 to 775 mμ[11]. The wavelength maxima and identification limits were reported for the reaction of 1,2-dinitrobenzene in alkaline solution, with cyclopentadiene, indene, benzfluorenes, and over 100 fluorene derivatives. *Table 1* lists the wavelength maxima and identification limits of a few compounds that react with 1,2-dinitrobenzene in the procedure. All that is known of the mechanism of reaction is that compounds with active hydrogen react with *o*- or *p*-disubstituted dinitroarenes in alkaline solution to give a blue to green chromogen. With the help of this test, 11*H*-indeno[1,2-*b*]quinoline[12] and the benzfluorenes[11] were detected in the urban atmosphere for the first time.

A thermochromic method for the detection of compounds containing the fluorenic CH_2 group is also available[13]. Negative results are obtained with

Table 1. Analysis for compounds containing the cyclopentadiene CH_2 group in the 1,2-dinitrobenzene procedure[11]

Compound	$\lambda_{max.}$, mμ	*Identification limit*, μg
Cyclopentadiene	570	1
Indene	650	0·6
Fluorene	695	0·1
2-Acetamidofluorene	680	1
2-Benzalaminofluorene	705	0·3
2-Acetylfluorene	705	0·4
2-Nitrofluorene	725	0·5
9-Carboxyfluorene	695	0·2
2,7-Diacetylfluorene	710	0·5
4*H*-Cyclopenta[*d,e,f*]phenanthrene	705	2
7*H*-Benzo[*c*]fluorene	740	2

fluorene derivatives containing electronegative groups or acidic hydrogen other than in the 9-methylene group. Thus, negative results could be expected with the amino, hydroxy, carboxy, acetyl, methanesulfonyl, and nitrofluorenes. The method has been used to confirm the presence of fluorene in urban atmospheres[14]. The postulated mechanism of the test is shown in *Figure 3.*

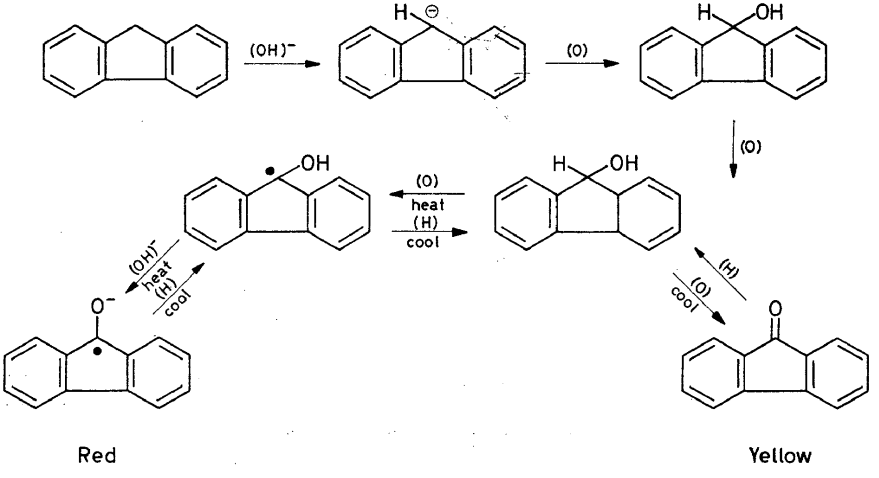


Figure 3. Reactions in the thermochromic detection of fluorenic compounds

Pyrene and many of its derivatives can be detected by a nitration test, illustrated in *Table 2*[15]. A brilliant blue polynitro anion, absorbing at approximately 620 mμ, is formed. Modification should make the procedure capable of detecting benzo[*a*]pyrene and some of its derivatives.

Benzo[*a*]pyrene can be detected[16] or estimated[17] fluorometrically as the cationic salt in the presence of other polynuclear aromatic hydrocarbons. In the detection method its fluorescence emission spectrum was

Table 2. Identification limit in nitration test[15]†

Pyrene derivative	*Identification limit*, μg	*Pyrene derivative*	*Identification limit*, μg
Pyrene	1	1-1′-Napthoyl-	10
1-Methyl-	5	1,2-Phthaloyl-	1
2-Methyl-	5	1-Nitro-	10
4-Methyl-	5	1,6-Dinitro-	1
1-Formyl-	2·5	1,3,6-Trinitro-‡	0·05
1-Acetyl-	5	1,3,6,8-Tetranitro-‡	0·01
1-Benzoyl-	10		

† Blue colour developed with all pyrene compounds except 1-acetyl- (blue-violet) and 1,3,6-trinitro- (green).
‡ Tested directly: 1 drop of Et_4NOH solution on paper, then 1 μl dimethylformamide solution of compound. By the test procedure, both compounds gave identification limit of 1 μg.

obtained in the presence of 50 other aromatic hydrocarbons. It has been estimated as the cationic salt after thin-layer chromatography; both the excitation and emission spectra can be obtained. Many other benzo[*a*]pyrene derivatives should also be capable of forming the fluorescent cation in sulfuric acid solution, and thus the functional group in this case would be the benzo[*a*]pyrene molecule. The fluorescence spectra of pure benzo[*a*]pyrene, by itself and in the presence of other hydrocarbons that could be found with it (following the alumina-column chromatographic separation of a complex mixture) is shown in *Figure 4*. The method can determine nanogram quantities of benzo[*a*]pyrene.

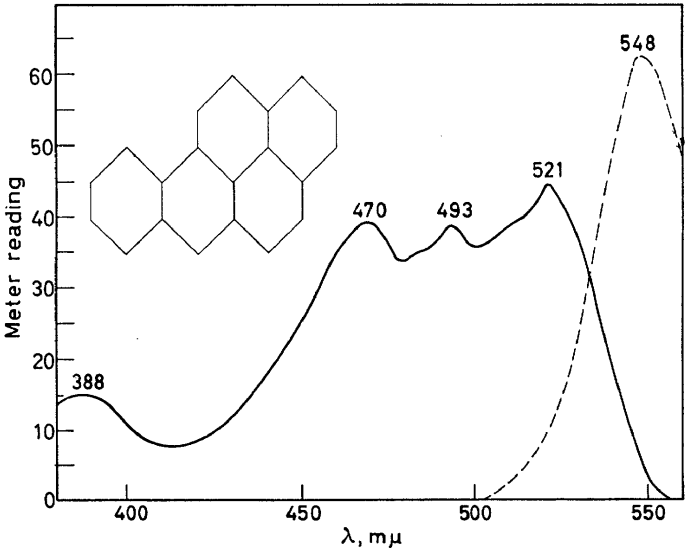


Figure 4. Fluorescence spectra of benzo[*a*]pyrene, 10^{-6}M, in concentrated sulfuric acid. Excitation spectrum at emission wavelength 548 mμ (——) and emission spectrum at excitation wavelength 520 mμ[17] (– – –).

Many new functional groups can now be detected or determined with the help of recently introduced methods of quenchofluorometric analysis[18, 19].

Polynuclear aromatic hydrocarbons containing the fluoranthene ring fluoresce in nitromethane solution, while other aromatic hydrocarbons are non-fluorescent[18]. The utility of this phenomenon is indicated by the fact that the fluorescence excitation and emission spectra of fluoranthene can

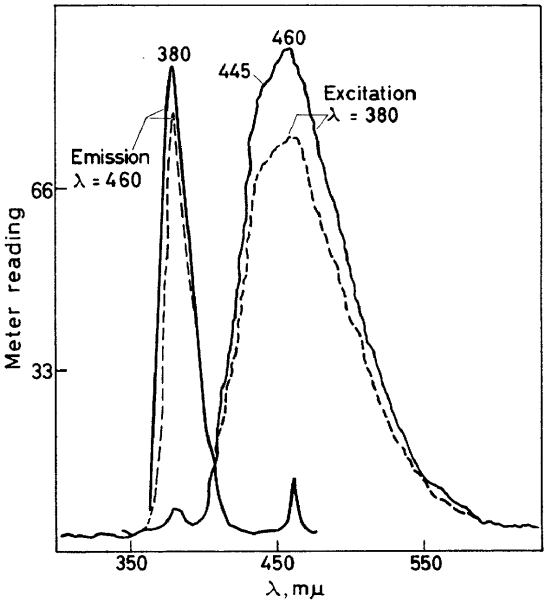


Figure 5. Fluorescence spectra of the pyrene fraction (——) and fluoranthene fraction of urban airborne particulates (– – –) both in nitromethane[18]

be obtained from the pyrene fraction of organic air-borne particles (*Figure 5*)[18]. The detection limits for obtaining the complete fluorescence excitation and emission spectra for the various fluoranthenic compounds are shown in *Table 3.* Through appropriate instrumental changes these limits could be

Table 3. Fluorometric detection limits for fluoranthic hydrocarbons in nitromethane[18]

Compound	*Identification limit,* ng/0·1 ml
Fluoranthene	340
Benzo[*b*]fluoranthene	400
Benzo[*k*]fluoranthene	13
Benzo[*g,h,i*]fluoranthene	250
Indeno[1,2,3-*c,d*]pyrene	14
Indeno[3,2-*j*]acenaphtho[1,2-*k*]fluoranthene	7
Diacenaphtho[1,2-*j*:1′,2′-*l*]fluoranthene	5
Acenaphtho[1,2-*b*]quinoxaline	750
Acenaphtho[1,2-*b*]benzo[*f*]quinoxaline	60
Acenaphtho[1,2-*b*]benzo[*g*]quinoxaline	70
Diacenaphtho[1,2-*b*:1′,2′-*d*]thiophene	100

decreased. By proper modification of the slits, and by use of a hand-picked, highly sensitive phototube and the most efficient vacuum tubes in the photomultiplier microphotometer, the sensitivity could be improved tenfold. Another improvement in sensitivity could be obtained by using 0·01-ml cells. It can be seen from *Table 3* that the presence of a pyrazine ring in the fluoranthenic part of the molecule did not quench the fluorescence. One molecule contained a ring system iso-π-electronic to fluoranthene (S substituted for —CH=CH—) and still fluoresced.

Another solvent system that can be used in the determination of fluoranthenic hydrocarbons is carbon disulfide[19]. Aza heterocyclic compounds fluoresce in carbon disulfide while the aromatic amines and polynuclear carbazoles thus far investigated do not. Carbon disulfide can also be used as the solvent in the fluorometric determination of perylene. The detection limit is 2 ng, so that perylene can be determined in commercial samples of benzo[*a*]pyrene, benzo[*e*]pyrene, and benzo[*g,h,i*]perylene. Hydrocarbons like anthracene, phenanthrene, chrysene, pyrene, benzo[*e*]pyrene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene, and coronene do not fluoresce. The procedure could probably be used in the analysis of perylene and many of its derivatives. Since the field of quenchofluorometry is in its infancy, other types of functional groups of value in analysis are likely to be found.

Quenching effects have also proved useful in thin-layer chromatography[18, 20]. Thus, benzo[*a*]pyrene and benzo[*k*]fluoranthene can be readily differentiated on an alumina thin-layer chromatogram developed with the aid of a volatile quencher (*Figure 6*)[18]. Many other examples have

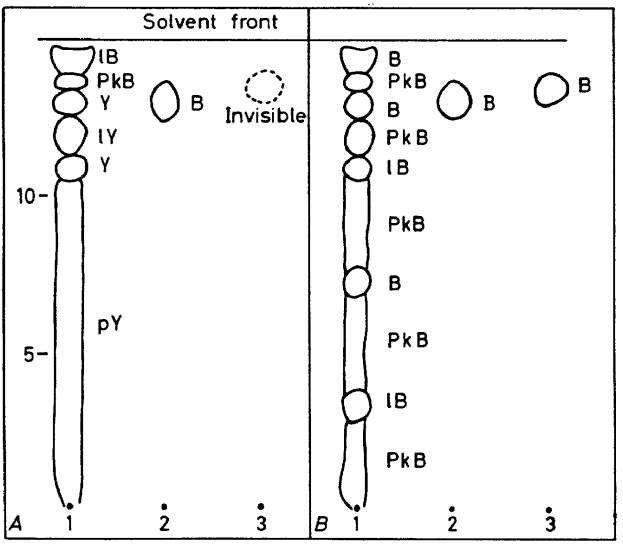


Figure 6. Alumina thin-layer chromatogram of (1) a benzene-soluble fraction of airborne particulates; (2) benzo[*k*]fluoranthene; (3) benzo[*a*]pyrene. *A*: Immediately after development with pentane–5 per cent 2-nitropropane; *B:* after plate has dried (about 30 min)[18]

been given of the application of the quenching phenomenon to differentiate between fluoranthenic hydrocarbons and other aromatic hydrocarbons and

between aromatic and aza heterocyclic aromatic hydrocarbons on the plate[20]. The use of a non-volatile quencher in the developer has also proved useful[20]. Thus, with pentane-nitrobenzene (9:1, V/V) as the developer on an alumina thin-layer plate, the fluorescence of the aromatic hydrocarbons and of some of the aza heterocyclic hydrocarbons is quenched. On treatment with trifluoroacetic acid fumes, practically all the aza compounds become fluorescent while the aromatic hydrocarbons remain non-fluorescent[20].

AZA HETEROCYCLIC COMPOUNDS

Another strange functional group that has been investigated is the pyridine group. The splitting of this ring into a polymethine dye has been used in determining pyridine in polluted air[21] and water[22, 23]. This type of reaction has been used to detect and determine pyridine and many of its derivatives. Examples of some of the pyridine derivatives that have been estimated by the ring-splitting reaction are given in *Table 4*. The 2-chloro, 2-methyl, 3-chloro, 3-cyano and 3-hydroxypyridines give negative results[24]. This reaction of the pyridine functional group needs much more thorough investigation, especially as to reagents and direct analysis on the paper or thin-layer chromatograms. The pentamethine dye obtained in the determination of pyridine with barbituric acid is red and gives an intense red fluorescence in alkaline solution[37]. In the presence of a magnesium salt the anion becomes blue. Neither the red fluorescence nor the blue colour has yet been thoroughly explored.

Pyridine derivatives have also been detected and estimated on paper chromatograms. 2-Ethylthioisonicotinamide is detected on a paper chromatogram by spraying with a 0·1 per cent solution of *p*-phenylenediamine followed by exposure to cyanogen bromide vapour[28]. A purple spot of the cationic dye is obtained in the presence of not less than 5 μg of 2-ethylthioisonicotinamide. Nicotinic acid in coffee was determined by paper chromatography followed by treatment of the spots first with cyanogen bromide vapour and then with benzidine reagent[38]. The density of the pink spots was determined with a photoelectric densitometer and the concentrations were calculated with the help of a standard curve. Pyridine derivatives have also been detected on paper chromatograms by spraying with aqueous barbituric acid followed by exposure to cyanogen chloride vapours[24].

The promising next step is the formation of a fluorescent chromogen from a pyridine functional group followed by direct fluorometric analysis of this spot on a paper or thin-layer chromatogram.

Another functional group of recent interest is obtained when a nitrogen atom is substituted for a methine group in a polynuclear aromatic hydrocarbon. These aza heterocyclic hydrocarbons can be detected or determined in the presence of many other types of organic compounds by quenched-fluorometric techniques[18, 19]. *Table 5* shows that the representatives of five classes of compounds are fluorescent in chloroform or alcohol. In solvents such as *o*-cresol, *N*,*N*-dimethylaniline and pyrrol the fluorescence of benz[*c*]acridine can be quenched while the others remain fluorescent. In nitromethane both benz[*c*]acridine and benzo[*k*]fluoranthene fluoresce while the others are quenched. In carbon disulfide, in nitrogen dioxide (68 per cent) in trifluoroacetic acid and in a solution of aluminium chloride

Table 4. Determination of pyridine compounds through the formation of a polymethane dye

Compound	*Halide reagent*	*Active hydrogen reagent*	$\lambda_{max.}$, mμ	*Reference*
Pyridine	Cyanogen chloride	Barbituric acid	578	24
Pyridine	Cyanogen chloride	Barbituric acid	578	25
Pyridine	Cyanogen bromide	Sulfanilic acid	465	26
Pyridine	Cyanogen bromide	Benzidine	520	27
2-Ethylpyridine	Cyanogen bromide	*p*-Phenylenediamine	460	28
Nicotinic acid	Cyanogen bromide	Aniline	420	29
Nicotinic acid	Cyanogen bromide	4′-Aminoacetophenone	420	30
Nicotinic acid	Phosphorus oxychloride	Phloroglucinol	632	31
Nicotinamide	Cyanogen bromide	Barbituric acid	550	32
N′-Methylnicotinamide	Cyanogen bromide	Barbituric acid	550	32
N′,*N*′-Diethylnicotinamide	Cyanogen bromide	Barbituric acid	560	33
Benzyl nicotinate	Cyanogen bromide	Sulfanilic acid	450	34
3-Pyridylcarbinol	Cyanogen bromide	4′-Aminoacetophenone	560	35
4-Picoline	Cyanogen chloride	Barbituric acid	598	24
Isonicotinic acid	Cyanogen chloride	Barbituric acid	615	24
Isonicotinylglycine	Cyanogen chloride	Barbituric acid	608	24
Isonicotinic acid hydrazide	Cyanogen chloride	Barbituric acid	600	36
2-Ethylthioisonicotinamide	Cyanogen bromide	*p*-Phenylenediamine	460	28

Table 5. Quenching effect of various solvents on five types of polynuclear compounds†

Solvent	*Benz*[c]-*acridine*	*Benzo*[k]-*fluoranthene*	*Benzo*[a]-*pyrene*	*4H-Benzo*[d,e,f]-*carbazole*	*1-Amino-pyrene*
Chloroform or alcohol	F	F	F	F	F
2,3-Butanedione	Q	Q	Q	Q	Q
Carbon disulfide	F	Q	Q	Q	Q
o-Cresol	Q	F	F	F	F
N,N-Dimethylaniline	Q	F	F	F	F
NO_2–trifluoroacetic acid (7:3)	F	Q	Q	Q	Q
Nitromethane	F	F	Q	Q	Q
$AlCl_3$ in nitromethane (10 per cent)	F	Q	Q	Q	Q
Pyrrol	Q	F	F	F	F

† F = Fluorescent; Q = quenched.

(10 per cent) in nitromethane, only benz[*c*]acridine fluoresces. This compound could be characterized through the quenching effect or through its fluorescence in appropriate quenching solvents. The quenchofluorometric technique has proved useful in the characterization of approximately 25 aza heterocyclic hydrocarbons separated and estimated with the help of column and thin-layer chromatographic analysis of coal tar pitch[39, 40], effluents from air pollution sources[40, 41], and urban atmospheres[42]. The use of quenchofluorometry in the determination of benz[*a*]acridine in the presence of other usually fluorescent substances is shown in *Figure 7.* This

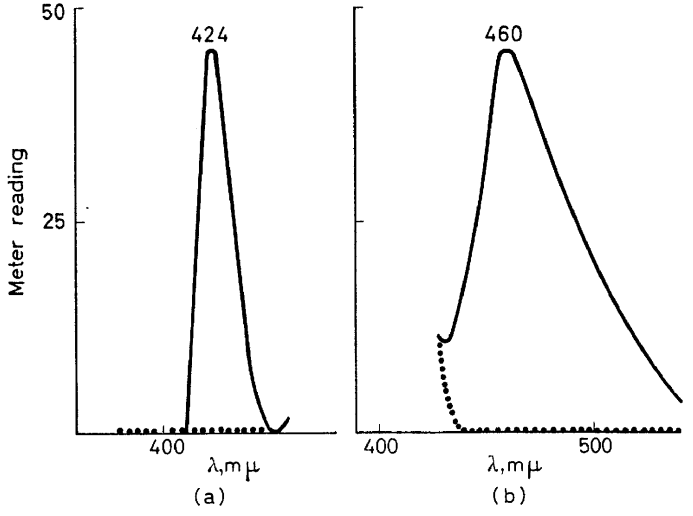


Figure 7. (*a*) Fluorescence excitation and (*b*) emission spectra of benz[*a*]acridine or a mixture of benzo[*a*]pyrene, benzo[*e*]pyrene, perylene, benzo[*k*]fluoranthene, and benz[*a*]acridine (——); and of a mixture of benzo[*a*]pyrene, benzo[*e*]pyrene, perylene, and benzo[*k*]fluoranthene (. . . .). All compounds are 2×10^{-5}M except benz[*a*]acridine which is 2×10^{-6}M. The solvent contains chloroform-*o*-nitrotoluene-trifluoroacetic acid (9:1:0·1, V/V)

Table 6. Fluorescence quenching effect† on an alumina thin-layer chromatogram[20]

	Quencher in developer					*Quenching after development*		
	Pentane-2-nitropropane (19:1)			*Pentane-nitrobenzene (19:1)*		CS_2–*TFA fumes*		NO_2–*TFA fumes*
Compounds	*TFA*←——*Wet*——→*Dry*			*Wet*——→*TFA*		*Before*	*After*	*After*
Acridine	G	Q	B	1G	G	B	BG	BG
Fluoranthene	1B	B	B	Q	Q	B	BG	Q
Pyrene	Q	Q	1B	Q	Q	BG	Q	Q
Benz[*a*]acridine	BG	Q	B	1G	BG	B	BG	BG
Benz[*c*]acridine	G	B	B	Q	G	B	G	G
Benzo[*a*]pyrene	Q	Q	1R	Q	Q	B	Q	Q
Benzo[*k*]fluoranthene	1B	B	B	Q	Q	B	B	Q
Pyrenoline	RO	B	B	Q	RO	1R	RO	RO
Benzanthrone	Y	Q	BG	Q	YG	BG	YG	dOY
1-Aminopyrene	Q	Q	B	Q	Q	B	Q	Q

† TFA = trifluoroacetic acid; B = blue; d = dull; G = green; l = light; O = orange; Q = quenched; R = red; Y = yellow.

technique is very useful in detecting and determining the aza heterocyclic compounds separated from complex mixtures.

The aza heterocyclic hydrocarbons (except indeno[1,2,3-*i*,*j*]isoquinoline) fluoresce in acidic nitromethane, while the fluorescence of the non-fluoranthenic aromatic hydrocarbons is quenched. In alkaline nitromethane these latter hydrocarbons are still non-fluorescent, but in addition some of the aza heterocyclic compounds are quenched, *e.g.*, benzo[*f*]quinoline, benzo[*h*]-quinoline, acridine, phenanthridine, acenaphtho[1,2-*b*]pyridine, benzo-[*l*,*m*,*n*]phenanthridine, benz[*a*]acridine, and pyrenoline. Thus, by a change in pH the method could distinguish between aza compounds, and the characterization of these compounds would be simplified. Examples of the use of this phenomenon in air pollution analysis are available[39].

The direct use of quenching in thin-layer chromatography has simplified the detection and identification of some two dozen aza heterocyclic hydrocarbons isolated from air pollution sources and urban air-borne particles[12, 39, 42]. Some examples of the use of a volatile quencher in the developer, a non-volatile quencher in the developer, and the fuming of a chromatogram with a volatile quencher have been given[20]. One use of these quenching methods is to differentiate aza compounds from other types of fluorescent molecules. *Table 6* presents a few examples of the various quenching techniques. Other examples will be given of quenching techniques that are highly selective for other types of functional groups.

AROMATIC AMINES AND HETEROCYCLIC IMINES

Compounds containing the functional group ArNH— can be detected and determined by means of the absorption and fluorescence spectra of the anion, ArN^-—[43]. Since these compounds are much less acidic than carboxylic acids or phenolic compounds, they will form anions only in strongly basic solutions. In this manner they are readily differentiated from the stronger acids. The absorption and fluorescence bands of the imine anion are at longer wavelengths than those of the neutral compound. This functional group can be sub-divided into two sub-groups on the basis of the fact that the aromatic primary amines and the aralkyl amines are much more basic than the diarylamines. Consequently the former class of compounds forms salts in moderately strong solutions, while the latter class does not. The absorption and fluorescence spectra of the aromatic amine salts then resemble that of the parent hydrocarbon, while the spectra of the diaryl amines are unchanged.

Use has been made of the absorption and fluorescence properties of the anions of the functional group Ar—NH— to prove the presence of carbazole and the polynuclear carbazoles in polluted air[44–46].

Another functional group that can be readily characterized on a thin-layer plate is the Ar—N—Ar grouping. Treatment with trifluoroacetic acid and ultraviolet light will give a blue to green colour with compounds such as carbazole, 9-methylcarbazole, diphenylamine, triphenylamine, and phenyl-1-naphthylamine[6]. Thus, in the presence of all types of fluorescent molecules, compounds containing the Ar—N—Ar grouping can be readily detected. An example of this phenomenon is the estimation of carbazole

in an air sample on a thin-layer plate. The organic particulate sample used in this case was obtained from air polluted with coal-tar-pitch fumes and was then separated by alumina thin-layer chromatography.

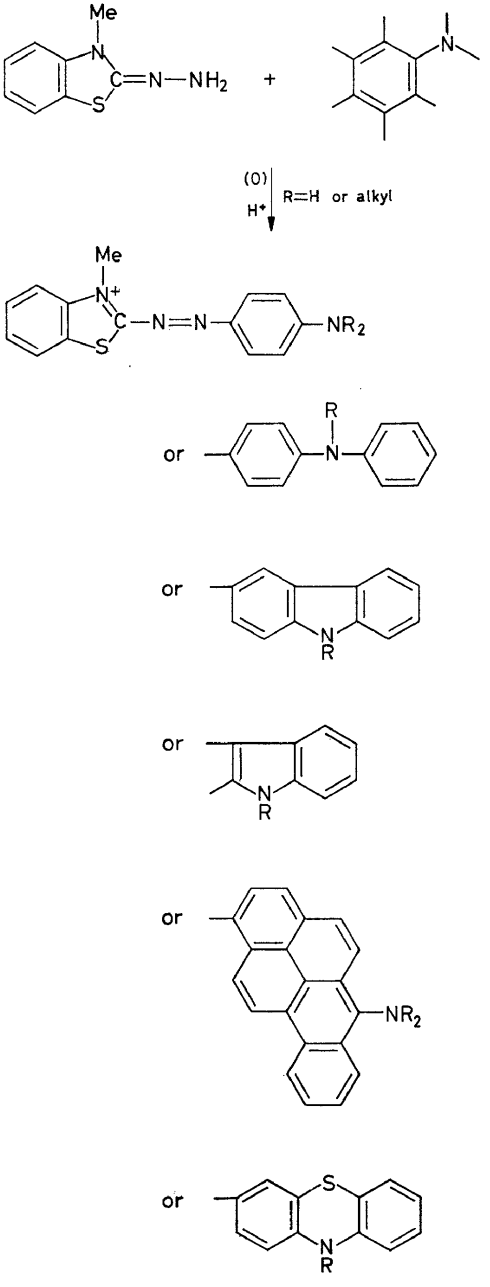


Figure 8. The functional group test with MBTH for C_6H_5—N— type compounds

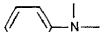
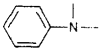
A functional group of the amine family that contains many more members is the  type. All kinds of compounds containing this grouping can be detected or determined with 3-methyl-2-benzothiazolinonehydrazone (MBTH)[47, 48]. Examples of the types of compounds that react are shown in *Figure 8*. The quantitative data obtained by reaction of MBTH with various compounds containing this functional group are given in *Table 7*. Since the method was developed with *N,N*-dimethylaniline as

Table 7. The 3-methyl-2-benzothiazolinone procedure for the determination of compounds containing the C_6H_5—N— grouping[47, 48]

Compound	$\lambda N_{max.}$, mμ	$\epsilon \times 10^{-3}$
Aniline	566	43
3,4-Dimethoxyaniline	570	32
N-Methylaniline	580	76
N,N-Dimethylaniline	598	84
N,N-Dimethyl-*p*-aminonitrosobenzene	630	72
	670	72
Diphenylamine	606	78
1-Naphthylamine	584	53
4-Nitro-1-naphthylamine	590	48
N,N-Dimethyl-2-aminofluorene	640	95
	670	97
1-Aminopyrene	755	42
Indole	508	32
9-Methylcarbazole	598	31
Phenothiazine	720	45
	765†	41
4-Phenylazoaniline	571	14
	644†	5
N-Methyl-4-phenylazoaniline	589	36
	630	34
	664†	32
N,N-Dimethyl-4-phenylazoaniline	604	70
	672	56
4-Phenylazo-1-naphthylamine	587	40
N,N-Dimethyl-4-aminostilbene	632	116
	667	119
N-(4-hydroxybenzylidene)aniline	571	54
	675	17
N,N-Dimethyl-β,β-dicyano-4-vinylaniline	637	56
	667	56
1-(*N,N*-Dimethyl-4-aminobenzylideneamino)pyrene	637†	90
	677	102
	755†	49

† Shoulder.

the standard, the molar absorptivities for most of the other compounds should be capable of improvement by modifications in the procedure. The detection limits for the compounds on the spot plate or on paper have also been determined. On paper these limits were slightly lower and were in the range 0·1–1 μg.

The  grouping can also be detected, identified, and

determined with the help of quenchofluorometry[43]. The method has been applied to polynuclear members of this family. *Table 8* shows that in alkaline aniline or dimethylhydrazine solution the aromatic amines and the heterocyclic imines fluoresce, while all the other types of compounds (except pyrenoline) are quenched. *Table 8* indicates many other analytical possibilities. The one pertinent to this section is the differentiation of the larger

Table 8. Selective quenching effect† of *o*-cresol and alkaline solutions of aniline and *N,N*-dimethylhydrazine[43]

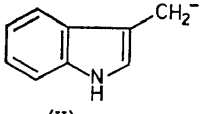
Compound	$CHCl_3$	*o-Cresol*	*TFA*‡	*Aniline*	*TEA*§	$Me_2N—NH_2$	*TEA*§
1-Naphthylamine	1B	Q	Q	B	G	B	G
1-Anthramine	G	G	B	G	1G	G	G
8-Aminofluoranthene	G	G	G	G	O	G	O
1-Aminopyrene	B	B	Q	B	G	B	G
Carbazole	1BV	Q	Q	1B	B	Q	B
4*H*-Benzo[*d,e,f*]-carbazole	B	Q	Q	Q	B	1B	B
7*H*-Dibenzo[*c,g*]-carbazole	B	Q	Q	Q	B	Q	B
Acridone	B	Q	Q	Q	B	Q	B
Pyrene	B	Q	Q	Q	Q	Q	Q
Benzo[*a*]pyrene	B	B	B	Q	Q	Q	Q
Fluoranthene	B	B	B	Q	Q	Q	Q
Benz[*c*]acridine	B	Q	Q	Q	Q	Q	Q
Pyrenoline	B	Q	Q	B	G	1B	1B
1,4-Dihydroxy-anthraquinone	Y	Q	Q	Q	Q	Q	Q

† B = Blue; G = green; l = light; O = orange; Q = quenched; Y = yellow.
‡ Drop of trifluoroacetic acid added to 1 ml of *o*-cresol solution.
§ Equal volume of 29 per cent methanolic tetraethylammonium hydroxide added to the anilineor *N,N*-dimethylhydrazine solution.

aromatic amines from the heterocyclic imines in *o*-cresol solution, in that the amines fluoresce while the carbazoles do not.

The usefulness of the quenching effects of various solvent systems in the direct fluorometric analysis on the plate of compounds containing the aromatic amine and diarylamine functional groups has been discussed.

Compounds containing the group

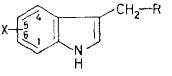


(II)

can be detected on a paper[49] or a thin-layer[50] chromatogram by treatment with acidified formaldehyde followed by heating at 90°C to dryness. Under ultraviolet light a positive result is indicated by the yellow to green fluorescent colour of the treated spot. The visible colour of the spots is yellow to brown. The fluorescence colours for the spots obtained on paper and thin-layer chromatograms and the detection limits obtained on the latter chromatograms are given in *Table 9*. On the thin-layer chromatogram indole

and its 3-acrylic acid were reported as giving a green fluorescent spot with the reagent at detection limits of 0·01 and 0·1 μg, respectively[50]. Negative results are reported with the paper chromatogram when the substituent on the methylene of the functional group is —OH, -indolyl or dimethylamine[49]. Since the method is very sensitive, the various indole compounds will be better characterized by direct spectrophotofluorometric examination of the spots under the various conditions at which optimum results are obtained[51].

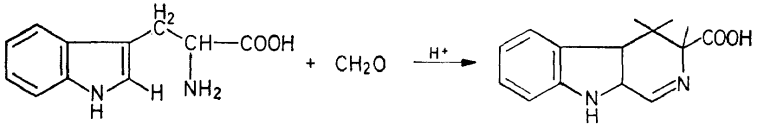
Table 9. Fluorescence identification on the chromatogram of the 3-methyleneindole functional group



X	R	*Fluorescence colour*†		*Identification limit, TLC*[50], μg
		PC[49]	*TLC*[50]	
H	—H	Y	Y	0·01
H	—OH	Neg.	Y	0·1
H	—CH_2OH	O	—	—
H	—COOH	Y	Y	0·005
H	—$CONH_2$	O	Y	0·01
H	—CN	O	Y	0·1
H	—$N(CH_3)_2$	Neg.	G	0·5
H	—CH_2COOH	Y	Y	0·01
H	—CH_2NH_2	O	Y‡	0·01
5-OH	—CH_2NH_2	Neg.	Br	0·01
H	—CH(OH)COOH	O	—	—
H	—$CH(NH_2)COOH$	O	Y‡	0·005
5-CH_3	—$CH(NH_2)COOH$	—	YG	0·005
5-OH	—$CH(NH_2)COOH$	—	Y	0·005
H	—CH_2CH_2COOH	O	Y	0·01

† PC = paper chromatogram; TLC = thin-layer chromatogram; Br = brown; G = green; O = orange; Y = yellow.
‡ With blue fluorescent border.

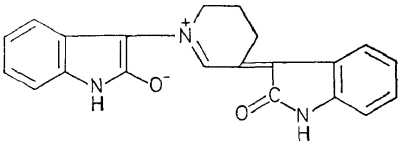
The procedure has been modified so that the 3-methylindoles give negative results below 20 μg concentrations while tryptophan can be determined fluorometrically on paper or thin-layer chromatograms at concentrations as low as 50 ng[52]. The equation for this test proceeds as follows:



Excitation λ=362 mμ Emission λ=530 mμ

Heterocyclic imines containing the functional group NH—CH_2—CH_2— in the ring react with various cyclic α-diketones and *o*-quinones to give coloured products related to isatin blue[53]. The method has been used to detect some piperidine and pyrrolidine alkaloids on a paper chromato-

gram[54]. Thus, the reaction between isatin and piperidine gives a blue dye(III), $\lambda_{max} = 635$ mμ and $\epsilon = 40{,}000$, in dimethylformamide[53].



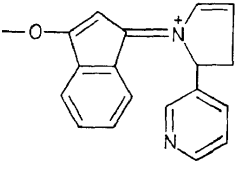
(III)

The results obtained with various compounds containing the functional group are shown in *Table 10*[54]. Some of these compounds lose a carboxy

Table 10. Reaction of some heterocyclic imines with isatin[54]

Compound	*Colour*
Proline	Blue
4-Hydroxyproline	Blue
3-Methylproline	Blue
4-Methylproline	Blue
Norhygrine	Blue
3-Hydroxypipecolic acid	Blue
4-Hydroxypipecolic acid	Brown
5-Hydroxypipecolic acid	Blue
Piperazine	Blue
Conhydrine	Blue-green
Coniine	Blue
iso-Pelletierine	Blue

group when they are baked with the reagent at 130°C on the paper chromatogram. A somewhat similar method has been used to determine nornicotine in tobacco and smoke[55]. The reagent, 1,3-indandione, reacts in a 1:1 mole ratio with compounds like pyrrolidine, piperidine, piperazine, morpholine, proline, and some aliphatic amines[55]. The postulatedchromogen (IV) obtained with nornicotine absorbs at 550 mμ.

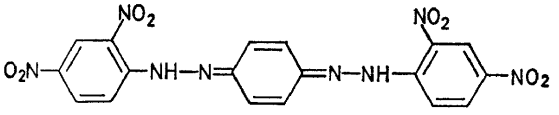


(IV)

QUINONES

Quinones can be divided into the following functional group types: benzoquinones, naphthoquinones, terminal-ring quinones, inner-ring *o*-quinones, and inner-ring *p*-quinones.

Benzoquinones can be determined by reaction with 2,4-dinitrophenylhydrazine[56]. The chromogen formed is stated to contain the structure:



(V)

Depending on the pH of the solution either the mono-anion or the di-anion of the chromogen could be determined. The di-anion has been determined[56]; it absorbs at a wavelength of 615 mμ. Napthoquinones and α-dicarbonyl compounds R—CO—CO—R (where R can be hydrogen or an alkyl or aryl substituent) also reacted in the procedure.

Terminal ring quinones are readily determined through their reaction with quinaldinium or lepidinium salts[57]. The determination of 1,4-naphthoquinone with 1-methylquinaldinium methosulfate is based on the reaction shown in *Figure 9.* Various benzoquinones, 1,2- and 1,4-naphtho-

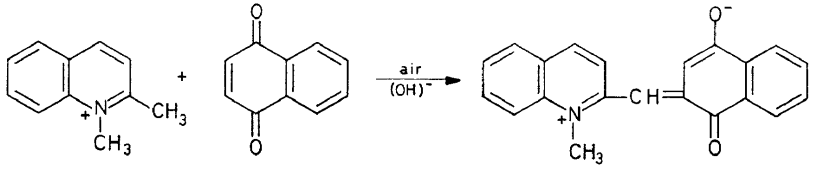


Figure 9. Determination of 1,4-naphthoquinone with 1-methylquinaldinium methosulfate[57]

quinones, and benz[*e*]acephenanthrylene-9,12-dione gave wavelength maxima in the range 650—715 mμ; *e.g.* with 1-methylquinaldinium methosulfate as the reagent, benzoquinone gave $\lambda_{max.} = 650$ mμ, $\epsilon = 43{,}000$, while 1,4-naphthoquinone gave $\lambda_{max.} = 660$ mμ, $\epsilon = 13{,}000$. Phenanthraquinone, anthraquinone, acenaphthaquinone, anthanthrone, benzanthrone, fluorenone, benzophenone, and benzalacetone gave negative results.

Table 11. Identification of 1,4-naphthoquinones with *o*-aminothiophenol[58]

Compound	*Colour*		*Identification limit,* μg
	Base	*Cation*	
Naphthoquinone	Red	Blue	0·1
2-Methylnaphthoquinone	Red	Blue†	0·05
2-Chloronaphthoquinone	Red	Blue	0·01
2,3-Dichloronaphthoquinone	Red	Blue	0·005
2,3-Dibromonaphthoquinone	Red	Blue‡	0·5

† Steam-heating the stain causes the colour to change to red; a drop of hydrochloric acid brings the blue colour back.
‡ A yellow-brown stain surrounded by a blue ring.

Methods for the detection[58] and determination[59] of 1,4-naphthoquinones have been described. The results obtained in the identification procedure are shown in *Table 11.* The equation for this striking test has been described[58]. The chromogen formed from 1,4-naphthoquinone has wavelengths at 495 and 790 mμ with $\epsilon = 16{,}000$ and 15,000, respectively[59]. The sensi-

tivity of this method is capable of further improvement. Negative results are given by benzoquinone, 1,2-naphthoquinone, phenanthraquinone, anthraquinone, acenaphthaquinone, and 1,4-naphthoquinones substituted with amino or hydroxyl groups in the 2 position. The *o*-aminothiophenol procedure should be tried with the larger polynuclear terminal ring *p*-quinones.

Inner-ring *o*-quinones can be identified or determined with the help of two tests[60]. In sulfuric acid they give blue to green colours with wavelength maxima ranging from that of 5,6-chrysenequinone ($\lambda = 585$ mμ and $\epsilon = 12{,}500$) to that of dibenz[*a*,*h*]anthra-5,6-quinone ($\lambda = 655$ mμ and $\epsilon = 10{,}500$). In the second test blue salts are formed by reaction of the quinones with 3,4-dimethoxyaniline in boiling acetic acid. The long wavelength bands range in wavelength and intensity from that of 9,10-phenanthraquinone ($\lambda = 590$ mμ and $\epsilon = 16{,}500$) to that of 9,10-retenequinone ($\lambda = 615$ mμ and $\epsilon = 12{,}300$). The methods have not as yet been applied to chromatograms for the characterization of these types of compounds.

Inner-ring *p*-quinones can be identified through the formation of the brilliantly coloured radical anion[61], whose formation equation is postulated in *Figure 10*. The usefulness of this procedure is shown by the test results

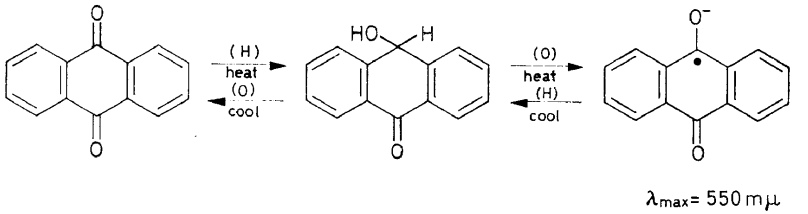


Figure 10. Equation for identification of inner-ring *p*-quinones through a thermochromic reaction

in *Table 12*[61]. With this procedure evidence was obtained that organic airborne particulates contain one or more inner-ring *p*-quinones with at least four fused rings per molecule. Negative results were obtained with benzoquinone, 1,4-naphthoquinone, phenanthraquinone, chrysene-5,6-dione, benzil, 9-acetylanthracene, and benzophenone.

Table 12. Thermochromic identification of some inner-ring *p*-quinones[61]

Compound	*Colour*†		*Identification limit,* μg
	Cold	*Hot*	
Anthraquinone	o	V	4
1-Aminoanthraquinone	y	R	6
2-Chloroanthraquinone	o	V	25
Naphthacene-5,12-dione	ro	B	5
Pentacene-6,13-dione	r	BG	35
Anthanthrone	y	B	8
Benz[*a*]anthracene-7,12-dione	y	B	20
Naphtho[2,3-*a*]pyrene-7,12-dione	g	B	6

† Capital letters signify intense colours; small letters signify light to pale colours. B = blue; G = green; O = orange; R = red; V = violet; Y = yellow.

p-HYDROXYSTYRYL FUNCTIONAL GROUP

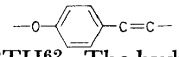
Of all the mono- and di-cyclic phenols only those containing the  group give a brilliant blue-green colour with MBTH[62]. The hydroxy group can be replaced by a methoxy, and probably by other types of RO— groupings. Examples of some of the compounds determined by this method are given in *Table 13*. The method could be used to differentiate iso-eugenol from eugenol and iso-safrole from safrole.

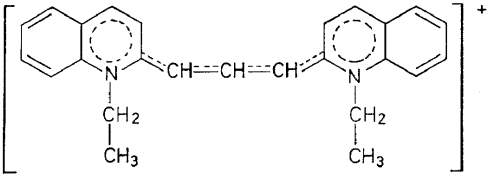
Table 13. Detection and determination of 4-hydroxystyrene derivatives with MBTH[62]

Compound	$\lambda_{max.}$, mμ	$\epsilon \times 10^{-3}$	*Identification limit*, μg
4-Methoxystyrene	667 634	62 61	0·1
4-Hydroxystilbene	667 624†	55 48	0·3
4-Methoxystilbene	667 624†	52 45	0·5
Isosafrole	667 624†	65 60	0·2
Isoeugenol	662 624†	64 58	0·08
3-Methoxy-4-hydroxypropenylbenzene	662 624†	62 57	0·08
4-Propenylveratrole	667 624†	70 63	0·08

† Shoulder.

FORMIC ACID AND FORMATES

Two main types of spectral procedures can be used for the analysis of formic acid[63]. The first involves reduction to formaldehyde and then the formation of a chromogen that can be measured colorimetrically, fluorometrically, or phosphorimetrically. The simplest procedure is to form a chromogen directly from the formic acid. Thus, by reaction with a quinaldinium or a lepidinium salt an intensely coloured trimethine dye can be formed. The structure of the blue cationic dye(VI) formed from 1-ethylquinaldinium iodide is as shown.



(VI)

The results obtained with different quinaldinium and lepidinium salts are shown in *Table 14*. Formaldehyde, acetaldehyde, propionaldehyde, glyoxal, benzaldehyde, dihydroxyacetone, acetic acid, malonic acid, citric acid, and ethyl formate give negative results in the procedure. To determine the formate esters, it is first necessary to hydrolyse them.

Table 14. Spectrophotometric determination of formate[63]

Reagent	*Procedure A*	
	$\lambda_{max.}$, mμ	$\epsilon \times 10^{-3}$
1-Ethylquinaldinium iodide	565	41
	610	91
1-Methylquinaldinium toluene-*p*-sulfonate	565	40
	610	85
1-Ethyllepidinium iodide	655	20
	710	81
1-Ethylquinaldinium toluene-*p*-sulfonate	565	29
	610	60
1-Benzyllepidinium chloride	650	7·5
	715	43
1-Methylquinaldinium methosulfate	565	15
	610	36
1-Ethyl-6-methylquinaldinium iodide	570	13
	617	25

ACYLATING AGENTS

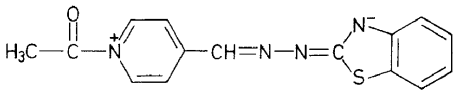
Acylating agents could be identified by the purple colour obtained with 4-pyridinecarboxaldehyde-2-benzothiazolylhydrozone on paper[64]. The

Table 15. Detection of acylating agents with 4-pyridinecarboxaldehyde-2-benzothiazolylhydrazone[64]

Compound	*Identification limit*, μg
Acetyl chloride	0·15
Chloroacetyl chloride	0·1
Dichloroacetyl chloride	0·5
Succinyl chloride	0·2
Phenylacetyl chloride	0·1
Nonanoyl chloride	0·06
α-Furoyl chloride	0·3
Benzoyl chloride	0·25
4-Nitrobenzoyl chloride	0·5
2-Ethoxybenzoyl chloride	0·1
Anisoyl chloride†	0·2
Cinnamoyl chloride	0·2
1-Naphthoyl chloride	0·1
4-Phenylazobenzoyl chloride	0·2
Acetic anhydride†	50
Methanesulfonic anhydride‡	1
p-Toluenesulfonyl chloride‡	7
p-Nitrobenzenesulfonyl chloride‡	20
Methanesulfonyl chloride†‡	7
Trichloromethanesulfonyl chloride‡	15
2-Bromoacetophenone	6
Cyanogen bromide†	0·4
Cyanuric chloride§	0·05
p-Nitrobenzyl bromide‖	2
2-Chloroacetophenone‖	30
Diphenylphosphoryl chloride†	30
1-Methyl-2-iodoquinolinium methosulfate† ¶	2

† Contact with triethylamine fumes gives best results for these compounds.
‡ 5–15 min required for colour to form.
§ Blue-green colour. Purple colours for other compounds.
‖ Drop test solution onto impregnated paper, heat 5 min at 100°C, add drop of triethylamine, and read colour.
¶ Test solvent is acetone containing 0·025 per cent water.

sensitivity of the reaction can be seen from *Table 15*. The chromogen obtained from acetyl chloride has the structure(VII):



(VII)

Under the conditions of the test the less reactive alkylating agents do not react. 4-Acetylpyridine-2-benzothiazolyhydrazone could be used also as the impregnated reagent. The identification limits are in the same range as those in *Table 15*, but the colours are bluer. Negative results were obtained with l-iodobutane, nonanoic acid, benzoic acid, trichloroacetylchloride, 3,5-dinitrobenzoyl chloride, thionyl chloride, trifluoroacetic anhydride, phthalic anhydride, maleic anhydride, and succinic anhydride. Apparently, electronegatively substituted acylating agents and ring-structured anhydrides do not give a positive purple colour in the test.

References

[1] E. Sawicki, T. W. Stanley, and T. R. Hauser. *Chemist-Analyst* **47**, 69 (1958).
[2] E. Sawicki, R. Miller, T. W. Stanley, and T. R. Hauser. *Anal. Chem.* **30**, 1130 (1958).
[3] E. Sawicki, T. W. Stanley, T. R. Hauser, H. Johnson, and W. C. Elbert. *Intern. J. Air Water Pollution* **7**, 57 (1963).
[4] E. Sawicki. *Chemist-Analyst* **47**, 9 (1958).
[5] E. Sawicki, T. W. Stanley, T. R. Hauser, and R. Barry. *Anal. Chem.* **31**, 1664 (1959).
[6] E. Sawicki and H. Johnson. *Mikrochim. Acta* **1964**, 435.
[7] K. H. Muller and H. Hoverlagen. *Deut. Apotheker-Ztg.* **100**, 309 (1960).
[8] E. Sawicki, T. W. Stanley, and W. C. Elbert. *Anal. Chem.* **33**, 1183 (1961).
[9] E. Sawicki, T. W. Stanley, and W. C. Elbert. *Microchemical Techniques* (Ed. N. Cheronis) Interscience, New York (1962).
[10] E. Stahl. *Deutsche Apotheker-Ztg.* **93**, 197 (1953).
[11] E. Sawicki, T. W. Stanley, and J. Noe. *Anal. Chem.* **32**, 816 (1960).
[12] E. Sawicki, S. McPherson, T. W. Stanley, J. Meeker, and W. C. Elbert. Presented at the Air Pollution Symposium of the 149th American Chemical Society Meeting, Detroit (1965).
[13] E. Sawicki and W. C. Elbert. *Chemist-Analyst* **48**, 68 (1959).
[14] E. Sawicki, W. C. Elbert, T. W. Stanley, T. R. Hauser, and F. T. Fox. *Anal. Chem.* **32**, 810 (1960).
[15] E. Sawicki and T. W. Stanley. *Chemist-Analyst* **49**, 77 (1960).
[16] E. Sawicki, T. R. Hauser, and T. W. Stanley. *Intern. J. Air Pollution* **2**, 253 (1960).
[17] E. Sawicki, T. W. Stanley, W. C. Elbert, and J. D. Pfaff. *Anal. Chem.* **36**, 497 (1964).
[18] E. Sawicki, T. W. Stanley, and W. C. Elbert. *Talanta* **11**, 1433 (1964).
[19] E. Sawicki, T. W. Stanley, and H. Johnson. Presented at the Air Pollution Symposium, 148th National Meeting of the American Chemical Society, Chicago, Ill. 1964, *Mikrochim. Acta* **1965**, 178.
[20] E. Sawicki, W. C. Elbert, and T. W. Stanley. *J. Chromatog.* **17**, 120 (1965).
[21] M. I. Tarenko. *Zh. Analit. Khim.* **11**, 644 (1956).
[22] R. C. Kroner, M. B. Ettinger, and W. A. Moore. *Anal. Chem.* **24**, 1877 (1952).
[23] E. Asmus and H. Garschagen. *Z. Anal. Chem.* **139**, 81 (1953).
[24] W. Nielsch and L. Giefer. *Z. Anal. Chem.* **171**, 401 (1960).
[25] E. Asmus and D. Papenfuss. *Z. Anal. Chem.* **185**, 201 (1962).
[26] H. Houben. *Brennstoff-Chem.* **44**, 299 (1963).
[27] R. Fabre, R. Truhaut, and M. Herbert. *Ann. Pharm. Franc.* **8**, 773 (1950).
[28] P. Kraus. *Cesk. Farm.* **12**, 246 (1963); *Anal. Abstr.* **11**, No. 2790 (1964).
[29] V. S. Mohan, B. L. Reid, and J. R. Couch. *J. Agr. Food Chem.* **7**, 42 (1959).
[30] E. B. Hale, G. K. Davis, and H. R. Baldwin. *J. Biol. Chem.* **146**, 553 (1942).
[31] G. Zinner and W. Deucker. *Naturwiss.* **49**, 300 (1962).
[32] O. Pelletier and J. A. Campbell. *J. Pharm. Sci.* **50**, 926 (1961).
[33] O. Pelletier and J. A. Campbell. *J. Pharm. Sci.* **51**, 594 (1962).
[34] L. Bors. *Studii Cerc. ştiinţ. Chim., Iaşi* **13**, 277 (1962); *Anal. Abstr.* **11**, 2797 (1964).

[35] E. G. Wollish, G. P. Kuhnis, and R. T. Price. *Anal. Chem.* **21**, 1412 (1949).
[36] W. Nielsch and L. Giefer. *Arzneimittel-Forsch.* **9**, 636 (1959).
[37] E. Asmus and H. Garschagen. *Z. Anal. Chem.* **138**, 404 (1953).
[38] H. Boddecker and A. R. Mishkin. *Anal. Chem.* **35**, 1662 (1963).
[39] E. Sawicki, T. W. Stanley, and W. C. Elbert. *Occupational Health Rev.* **16**, No. 3, 8 (1964).
[40] E. Sawicki, J. Meeker, and M. Morgan. Presented at the Air Pollution Symposium of the 149th American Chemical Society Meeting, Detroit (1965).
[41] E. Sawicki, T. W. Stanley, and W. C. Elbert. *J. Chromatog.* in the press (1965).
[42] E. Sawicki. Presented at the Eastern Analytical Symposium, New York (1964).
[43] E. Sawicki and H. Johnson. Presented at the International Symposium on Microchemical Techniques, Pennsylvania State University (1965).
[44] E. Sawicki, T. R. Hauser, T. W. Stanley, W. C. Elbert, and F. T. Fox. *Anal. Chem.* **33**, 1574 (1961).
[45] D. F. Bender, E. Sawicki, and R. M. Wilson, Jr. *Anal. Chem.* **36**, 1011 (1964).
[46] D. F. Bender, E. Sawicki, and R. M. Wilson, Jr. *Intern. J. Air Water Pollution* **8**, 633 (1964).
[47] E. Sawicki, T. W. Stanley, T. R. Hauser, W. C. Elbert, and J. L. Noe. *Anal. Chem.* **33**, 722 (1961).
[48] E. Sawicki, T. R. Hauser, T. W. Stanley, W. C. Elbert, and F. T. Fox. *Anal. Chem.* **33**, 1574 (1961).
[49] Z. Prochazka. *Chem. Listy* **47**, 1643 (1953).
[50] E. Stahl and H. Kaldewey. *Z. Physiol. Chem.* **323**, 182 (1961).
[51] E. Sawicki, T. W. Stanley, and H. Johnson. *Microchem. J.* **8**, 257 (1964).
[52] T. W. Stanley and E. Sawicki. *Anal. Chem.*, in the press.
[53] A. W. Johnson and D. J. McCaldin. *J. Chem. Soc.* **1957**, 3470.
[54] D. J. McCaldin. *Can. J. Chem.* **38**, 1229 (1960).
[55] E. Glock and M. P. Wright. *Anal. Chem.* **35**, 246 (1963).
[56] D. P. Johnson, F. E. Critchfield, and J. E. Ruch. *Anal. Chem.* **34**, 1389 (1962).
[57] E. Sawicki, T. W. Stanley, and T. R. Hauser. *Anal. Chim. Acta* **21**, 392 (1959).
[58] E. Sawicki and W. C. Elbert. *Anal. Chim. Acta* **23**, 205 (1960).
[59] E. Sawicki, T. W. Stanley, and S. McPherson. *Chemist-Analyst* **50**, 103 (1961).
[60] E. Sawicki and W. C. Elbert. *Anal. Chim. Acta* **22**, 448 (1960).
[61] E. Sawicki, T. W. Stanley, and T. R. Hauser. *Anal. Chem.* **30**, 2005 (1958).
[62] E. Sawicki, T. R. Hauser, and S. McPherson. *Chemist-Analyst* **50**, 68 (1961).
[63] E. Sawicki, T. W. Stanley, J. Pfaff, and J. Ferguson. "Analytical Chemistry 1962." *Proceedings of Feigl Anniversary Symposium, Birmingham, England*, Elsevier, Amsterdam (1963).
[64] E. Sawicki, D. F. Bender, T. R. Hauser, R. M. Wilson, Jr., and J. E. Meeker. *Anal. Chem.* **35**, 1479 (1963).