THE FLUORESCENCE-QUENCHING EFFECT IN THIN-LAYER CHROMATOGRAPHY OF POLYNUCLEAR AROMATIC HYDROCARBONS AND THEIR AZA ANALOGS

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INTRODUCTION

Polynuclear aromatic hydrocarbons show vivid fluorescence on thin-layer chromatograms[1,2]. The detection limits range from about 1 to 1000 ng and average[2] about 10 ng. Consequently the fluorescent colors are of practical value in separating trace amounts of these compounds from extremely complicated mixtures, such as organic airborne particulates. Other polynuclear aromatic compounds, such as aldehydes, ketones, amines, and oxygen, sulfur, aza and imino heterocyclic compounds also show fluorescent colors of the same order of intensity on thin-layer chromatograms[2]. The detection limits for the aza heterocyclic compounds average around 3 ng, and most of the compounds can be detected at 1 ng or 0.001 μg.

It was believed that since the polynuclear compounds are so intensely fluorescent, any method that could selectively quench some of these groups of compounds would be invaluable in group characterization through thin-layer and paper chromatographic studies. Since organic atmospheric and air pollution source particulates contain an extremely large number of unknown fluorescent molecules[3], the quenching effect should help in the characterization of these unknowns.

EXPERIMENTAL

Materials

All polynuclear aromatic compounds were obtained from commercial sources.

Equipment

The fluorescent colors were examined and sprayed in a Chromato-Vue cabinet (Kensington Scientific Corp., Berkeley 10, Calif.) under a 3660 Å light source.

Solvent systems

Pentane–ether (19:1, v/v); pentane–2-nitropropane (19:1, v/v); pentane–2-nitropropane (9:1, v/v); pentane–nitrobenzene (9:1, v/v).

Fuming reagents

Trifluoroacetic acid, nitromethane, carbon disulfide.

Method of development

Smooth glass plates (20 × 20 cm) were coated with 250 μ thickness of alumina with an applicator. The development was performed by the ascending method in the usual procedure.

Fuming methods

Trifluoroacetic acid test. The line of development is treated with small bursts of trifluoroacetic acid fumes. A throwaway pipet fitted with a squeeze bulb or a closed tank containing the plate and trifluoroacetic acid fumes can be used. Any change in fluorescence color is noted.

Other fuming reagents used in the procedure were nitromethane, carbon disulfide, and nitrogen dioxide–trifluoroacetic acid (3:10). Nitromethane works best as a fine liquid spray.

DISCUSSION

Thin-layer chromatography of aza compounds

One factor common to all the separations of aza compounds on alumina, whether with pentane–nitrobenzene (9:1, v/v) or pentane–2-nitropropane (3:1, v/v), is that these substances are separated according to their size and the amount of steric hindrance around the aza nitrogen atom. The adsorbent has a very strong attraction for the aza nitrogen atom. For this reason the following isomers are readily separated: benzo(*f*)quinoline and benzo(*h*)quinoline, benz(*a*)acridine and benz(*c*)acridine, 7-phenyldibenz(*c,h*)acridine and 14-phenyldibenz(*a,j*)acridine. Characteristics of these and other compounds are presented in Table I. The R_F values varied with

TABLE I

R_F VALUES AND FLUORESCENCE COLORS OF SOME AZA HETEROCYCLIC COMPOUNDS ON ALUMINA

Compound	Pentane–nitrobenzene (9:1, v/v)			Pentane–2-nitropropane–triethylamine (9:1:0.01)			
	R_F	Color*		R_F	Color*		
		Wet plate	After TFA		Wet plate	Dry plate	After TFA
7-Phenyldibenz(*c,h*)acridine	0.94	—**	YG	0.93	BG	BG	YG
Benz(*c*)acridine	0.92	—	G	0.92	—	B	G
Pyrenoline	0.45	—	RO	0.82	B	B	RO
Acridine	0.37	1G	G	0.66	—	B	G
Benzo(*h*)quinoline	0.35	—	B	0.90	—	B	B
Benzo(*f*)quinoline	0.30	—	B	0.53	—	B	B
14-Phenyldibenz(*a,j*)acridine	0.28	G	G	0.67	B	B	B
Indeno(1,2,3-*i,j*)isoquinoline	0.26	1B	dRO	0.65	YG	YG	R
Benz(*a*)acridine	0.23	1G	BG	0.61	B	B	BG
Acenaphtho(1,2-*b*)pyridine	—	—	—	0.59	B	B	BG
Benzo(*l,m,n*)phenanthridine	0.15	—	G	0.48	B	B	G

* B = blue; G = green; O = orange; Pk = pink; R = red; Y = yellow; d = dull; l = light.
** — = quenched.

changes in the amount of water on the alumina and with varying batches of alumina. Standards should be run automatically on the plate with any unknown material that is being investigated.

Pyrenoline is readily detected on the plate after separation followed by about 5 min of irradiation under the 3660 Å light source of the Chromato-Vue cabinet. A visible violet color is obtained, with a detection limit of ~ 0.01 μg of pyrenoline. Acid interferes in this characterization test. Thus, through the ordinary procedure of separation and examination of the plate under ultraviolet light, pyrenoline can be selectively differentiated from all the other aza heterocyclic compounds.

Direct quenching

With a developing mixture consisting of pentane–nitrobenzene (9:1, v/v), aromatic hydrocarbons are separated near the top of the plate but cannot be seen under ultraviolet light because their fluorescence has been quenched. Compounds such as anthracene, phenanthrene, pyrene, chrysene, fluoranthene, benz(a)anthracene, benzofluorenes, benzopyrenes, perylene, benzo(k)fluoranthene, anthanthrene, and dibenzopyrenes are non-fluorescent on such a plate. In addition benzo(f)quinoline, benzo(h)quinoline, pyrenoline, and 7-phenyldibenz(c,h)acridine are quenched. At higher concentrations the latter compound will appear as a blue-green fluorescent
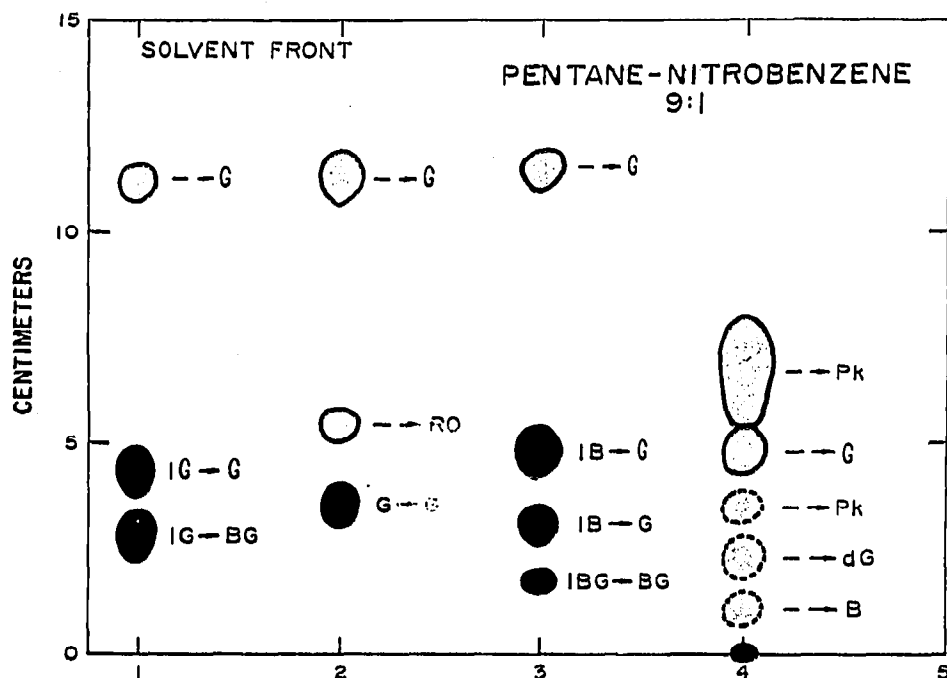

Fig. 1. Thin-layer chromatogram on alumina with pentane–nitrobenzene (9:1, v/v) as developer. All compounds were present in 2-μg amounts. Reading from top to bottom: (1) Benz(c)acridine, acridine and benz(a)acridine. (2) 7-Phenyldibenz(c,h)acridine, pyrenoline and 14-phenyldibenz-(a,j)acridine. (3) A basic fraction obtained from an airborne particulate sample in which the main source of pollution was coal tar pitch fumes. (4) A benzene-soluble fraction of urban airborne particulates. The notation —— ——→ G signifies that the compound was not fluorescent on the wet plate but on treatment with trifluoroacetic acid fumes it fluoresced with a green color. The following compounds were also separated on this plate and were non-fluorescent on the wet plate or after treatment with trifluoroacetic acid fumes: anthracene, phenanthrene, pyrene, benzo(a)-pyrene, benzo(e)pyrene, fluoranthene, benzo(k)fluoranthene and acenaphtho(1,2-b)pyridine. For color abbreviations see footnote * to Table I.

spot. The last two compounds become fluorescent when they are treated with trifluoro-acetic acid fumes. An example of such a separation is shown in Fig. 1. The polycyclic aromatic hydrocarbons do not show under ultraviolet light, whereas most of the aza heterocyclic derivatives do. With trifluoroacetic acid fumes some of the non-fluorescent aza heterocyclic compounds become fluorescent. Thus by this system mixtures containing both aromatic and aza hydrocarbons can be readily separated and the latter derivatives can be more readily characterized. Some of the other types of aromatic compounds that have been found to be non-fluorescent in this type of system are 1-aminopyrene, 2-aminoanthracene, carbazole, the benzocarbazoles, 1-acetyl-pyrene, salicylaldehyde and benzanthrone. This system of separation and characterization appears to be highly selective for many of the larger aza heterocyclic hydrocarbons.

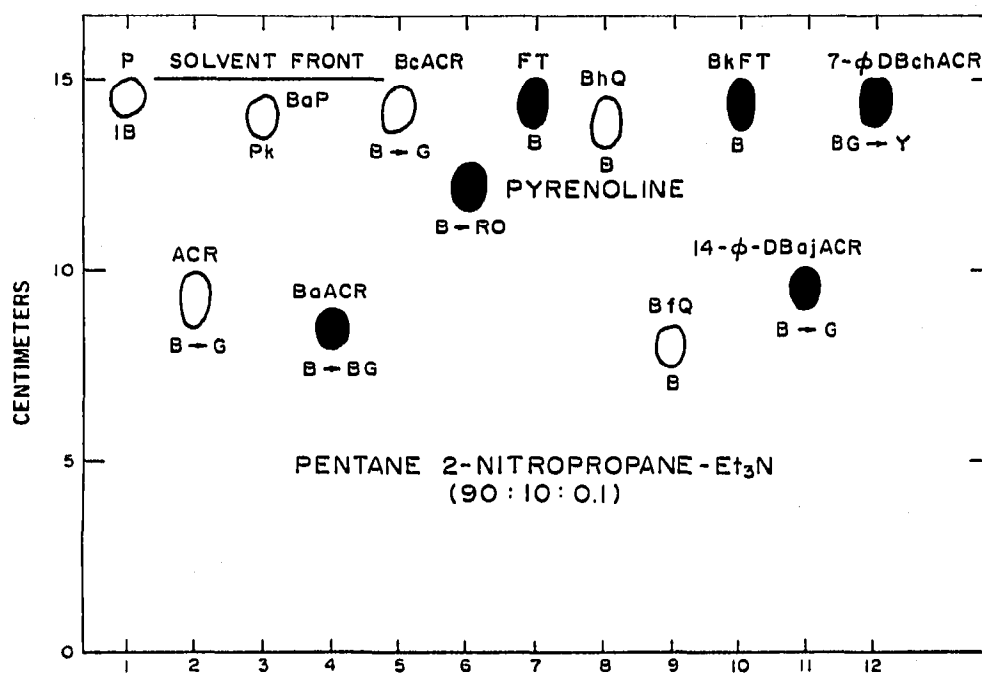A second way of taking advantage of the quenching effect in thin-layer chro-

Fig. 2. Thin-layer chromatogram on alumina with pentane–2-nitropropane–triethylamine (9:1: 0.01, v/v) as developer. (1) pyrene; (2) acridine; (3) benzo(a)pyrene; (4) benz(a)acridine; (5) benz-(c)acridine; (6) pyrenoline; (7) fluoranthene; (8) benzo(h)quinoline; (9) benzo(f)quinoline; (10) benzo(k)fluoranthene; (11) 14-phenyldibenz(a,j)acridine; (12) 7-phenyldibenz(c,h)acridine. Darkened spots are fluorescent on the wet or dry plate. Light spots are non-fluorescent on the wet plate and become fluorescent after approximately 1 h of standing. Spots on dry plate show no change in fluorescent color with trifluoroacetic acid fumes except as shown after an arrow. For color abbreviations see footnote * to Table I.

matography is by using a low-boiling quenching solvent in the developer. The evaporation should be just fast enough that the plate will be wet with this quencher for about half an hour. Two readings of the plate are made: one while it is wet and one while it is dry. In this way the quenched and non-quenched spots can be differentiated and found on the plate. An example of this type of differentiation is shown in Fig. 2. Non-fluoranthenic polycyclic hydrocarbons are non-fluorescent on a fresh plate, e.g. anthracene, pyrene, chrysene, the benzopyrenes, perylene, anthanthrene,

etc. Fluoranthenic hydrocarbons such as fluoranthene and benzo(*b*)- and benzo(*k*)-fluoranthene are fluorescent on the wet or dry plate. Some of the aza heterocyclic hydrocarbons are fluorescent and some are not. Once the plate is dry, however, all spots fluoresce. Trifluoroacetic acid fumes can bring out the fluorescence of all the aza compounds on the fresh plate.

For this kind of system the developer is an alkane containing 5–10 % of a nitroalkane. If a little acid is present in the developing solution instead of the triethylamine, then development is somewhat similar but all aza and fluoranthenic compounds are fluorescent on the wet plate under ultraviolet light.

Indirect quenching

A third fluorescence quenching method of value in thin-layer chromatography consists of treating a chromatogram with the fumes of a low boiling-quencher, *e.g.*
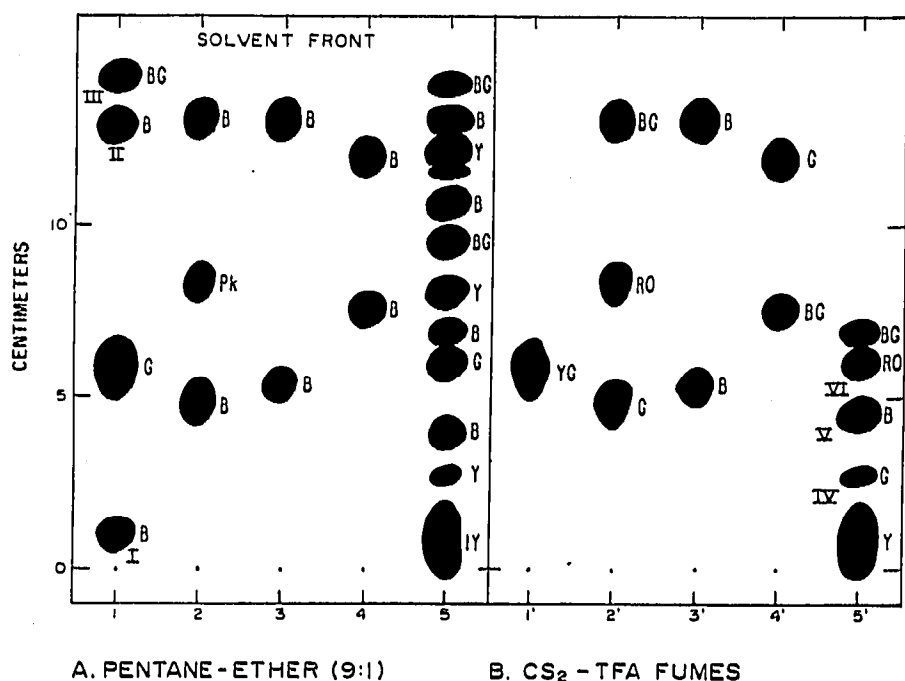

A. PENTANE-ETHER (9:1)   B. CS₂ - TFA FUMES

Fig. 3. Thin-layer chromatogram on alumina with pentane–ether (9:1, v/v) as developer. All compounds were present in approximately 2 μg amounts. Reading from top to bottom: (1) Pyrene, benzo(*a*)pyrene, benzanthrone and 1-aminopyrene. (2) Fluoranthene, pyrenoline and benz(*a*)-acridine. (3) 7-Phenyldibenz(*c,h*)acridine and benzo(*f*)quinoline. (4) Benz(*c*)acridine and acridine. (5) Benzene-soluble fraction of urban airborne particulates. (A) Read under ultraviolet light after development; (B) then spray with fumes of carbon disulfide saturated with trifluoroacetic acid. For color abbreviations see footnote * to Table I.

nitromethane, carbon disulfide or nitrogen dioxide. An example of this quenching effect is shown in Fig. 3. Here the quenching fumes consist of carbon disulfide saturated with trifluoroacetic acid. Compounds such as 1-aminopyrene (I), benzo(*a*)pyrene (II) and pyrene (III) are quenched. Carbon disulfide is a more potent quencher, for in addition to quenching spots I, II and III it also quenches benzo(*f*)quinoline. Nitromethane spray or nitrogen dioxide–trifluoroacetic acid fumes will also quench many non-fluoranthenic polycyclic aromatic hydrocarbons. With a nitroalkane or a carbon disulfide quencher the fluorescence of non-fluoranthenic hydrocarbons, polycyclic

carbazoles, many polycyclic aromatic amines, and many aromatic carbonyl derivatives can be quenched on a thin-layer plate. It is even possible to quench some of the aza heterocyclic compounds on the plate with freshly distilled nitromethane. Thus, acridine, the benzacridines and pyrenoline can be quenched while the fluoranthenic hydrocarbons still fluoresce. For this quenching to take place, the spot must be saturated only with nitromethane, and acid must be absent.

With the help of trifluoroacetic acid fumes all the aza compounds can be made to fluoresce as salts. Thus, the aza compounds can be distinguished from the fluoranthenic hydrocarbons, since with acid they become fluorescent, their fluorescence intensifies, or the fluorescent color changes. Additional evidence by which an aza hydrocarbon can be distinguished from an aromatic hydrocarbon is obtained by fuming the spots with nitrogen dioxide–trifluoroacetic acid (7:3, v/v) fumes. The aromatic hydrocarbons are quenched, while most of the aza hydrocarbons remain fluorescent. For example, pyrene, fluoranthene, benzo(*a*)pyrene, perylene and benzo-(*k*)fluoranthene are quenched while indeno(1,2,3-*i,j*)isoquinoline, dibenz(*a,j*)acridine and benzanthrone have dull yellow-brown, dull gray-blue and dull orange-yellow fluorescence, respectively. Acridine, benzo(*f*)quinoline, benzo(*h*)quinoline, benz(*a*)-acridine, benz(*c*)acridine, acenaphtho(1,2-*b*)pyridine, benzo(*l,m,n*)phenanthridine and pyrenoline are brilliantly fluorescent under these conditions. On the basis of the work in this paper it is believed that highly selective quenchers for diverse groups of compounds can be developed and should prove extremely useful in paper and thin-layer chromatographic separation of fluorescent compounds.

APPLICATION

The separation of a basic fraction obtained from polluted air is shown in Fig. 1. The presence of aza compounds can be inferred from the presence of the fluorescent spots. Work on the identification of these spots is proceeding.

The separation of a benzene-soluble fraction of an urban airborne particulate sample is shown in Fig. 3A. The majority of the spots are quenched with carbon disulfide–trifluoroacetic acid. A new spot, V, appears in Fig. 3B. The emerald green spot at IV and the red-orange spot at VI are common to the organic benzene-soluble fractions of urban airborne particulates. These basic compounds are invaluable as markers.

SUMMARY

Fluorescence quenching effects were studied with volatile quencher in the developer, with a relatively non-volatile quencher in the developer, and with a volatile quencher after development. On the basis of quenching effects the aromatic compounds can be divided for analytical purposes into non-fluoranthenic aromatic, fluoranthenic and aza hydrocarbons. The latter group can be further subdivided.

Two systems for the separation of polycyclic aza hydrocarbons elute according to the size of the ring system and the amount of steric hindrance at a ring nitrogen atom.

Pyrenoline can be characterized by the specific violet color it forms on the plate (identification limit = 0.01 μg).

The various methods show promise for use in air pollution studies.

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