Intramolecular Fluorescence Quenching of Anthracene by Heterocyclic Ligands

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The 9-anthryl and 9-anthrylmethyl groups and their 10-methyl derivatives have been used to form quaternary salts of some pyridines and *N*-methylimidazole. The imidazolium compounds exhibit normal anthracenic fluorescence but the pyridinium materials show efficient intramolecular quenching. An electron transfer mechanism is advanced which accounts for these phenomena.

OVER the last few years, considerable interest has been shown in the processes by which the fluorescence of aromatic hydrocarbons with low lying singlet states is quenched by molecules which have a first excited singlet state of higher energy. Anthracene fluorescence, for instance, is quenched by the pyridinium ion [1] in both inter- and intra-molecular processes. Barnett and Cook [2] noted the non-fluorescence of (Ic) in 1921, but mechanistic studies have been undertaken only recently, when Hann *et. al.*[1] showed that the intermolecular fluorescence quenching of 9-methylanthracene (Ib) by pyridinium ion is a diffusion-controlled phenomenon. They ascribed the lack of emission from (If) to ' a quasi-free interaction of the attached pyridinium moiety '. Ermolaev *et al.*[3] have suggested that such an intermolecular interaction consists of two stages. The first involves transfer of a proton from excited anthracene to the pyridinium group, which facilitates the subsequent transfer of an electron from the anthracenyl anion to the pyridinium cation. This idea appears of questionable relevance to the present situation. The pyridinium ion already has a good electron-accepting capability as established by its polarographic reducibility.[4] Consequently it seems unnecessary to postulate a thermodynamically unfavourable proton transfer or hydrogen bond formation as an initial prerequisite for electron transfer. Furthermore, Nakajima and Akamatu [5] have shown that anthracene

fluorescence can be quenched intermolecularly by electron acceptors, and that the quenching rate constant is a function of the electron affinity of the acceptor.

We have investigated the intramolecular quenching properties of two types of heterocyclic cationic groups, pyridinium and *N*-methylimidazolium ions linked to the anthracene chromophore both directly and *via* a methylene bridge. On the one hand, the π-deficient pyridinium ions provide contrasting electron accepting ability to the π-excessive *N*-methylimidazolium system, which should help to clarify the mechanism of quenching. On the other, these compounds provide model systems for the analysis of the fluorescence characteristics of polycyclic aromatic hydrocarbons covalently bonded to nucleic acid bases.[6,7]

RESULTS AND DISCUSSION

The heterocyclic salts were prepared by standard methods involving quaternisation of the tertiary amine by a suitable halogen derivative of the aromatic hydrocarbon. In some cases, for instance 2- and 4-methoxypyridinium compounds, the products proved to be insufficiently stable for spectroscopic examination since they readily solvolysed in polar solvents. Characteristically, such materials on recrystallisation from alcohol yielded the corresponding ethoxy- or ethoxymethylanthracenes. Most of the compounds described in the Table were virtually insoluble in non-hydroxylic solvents,

[1] R. A. Hann, D. R. Rosseinsky, and T. P. White, *J.C.S. Faraday II*, 1974, 1522.

[2] E. de B. Barnett and J. W. Cook, *J. Chem. Soc.*, 1921, 901.

[3] V. Ermolaev, A. Krasheninnikov, and A. V. Shablya, *Optics and Spectroscopy*, 1973, **34**, 564.

[4] S. G. Mairanovsky, ' Catalytic and Kinetic Waves in Polarography,' Plenum Press, New York, 1968.

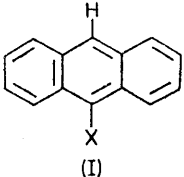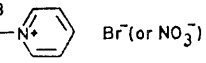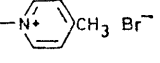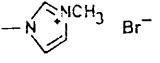[5] A. Nakajima and H. Akamatu, *Bull. Chem. Soc. Japan*, 1968, **41**, 1961.
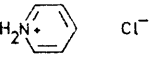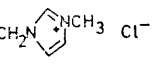
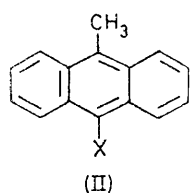[6] G. M. Blackburn, R. G. Fenwick, and M. H. Thompson, *Tetrahedron Letters*, 1972, 589.

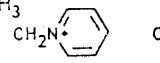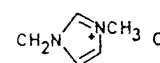[7] M. Kodama and C. Nagata, *Biochemistry*, 1975, **14**, 4645.

and n.m.r. spectra were obtained with difficulty or not at all [(Ic—e) and (IIb)].

The compounds which proved to be sufficiently stable were fully characterised and their spectroscopic proper-

Fluorescence quantum yields in ethanol at room temperature

	Compound	Quantum yield
(I) structure with X, H at 9-position		
a; X = H	(Ia)	0.27 [a]
b; X = CH$_3$	(Ib)	(0.29 [b])
c; X = —N$^+$(pyridyl) Br$^-$ (or NO$_3^-$)	(Ic)	N.F. [c]
d; X = —N$^+$(pyridyl)CH$_3$ Br$^-$	(Id)	N.F.
e; X = —N(imidazolyl-NCH$_3$) Br$^-$	(Ie)	0.45
f; X = CH$_2$N$^+$(pyridyl) Cl$^-$	(If)	N.F.
g; X = CH$_2$N(imidazolyl-NCH$_3$) Cl$^-$	(Ig)	0.46
h; X = anthracen-9-yl	(Ih)	0.31
(II) structure with CH$_3$ at 9-position, X at 10		
a; X = CH$_3$	(IIa)	0.62
b; X = CH$_2$N$^+$(pyridyl) ClO$_4^-$	(IIb)	0.005 [d]
c; X = CH$_2$N(imidazolyl-NCH$_3$) ClO$_4^-$	(IIc)	0.63

[a] Anthracene was used as the reference compound.[8-10]
[b] Ref. 10. [c] N.F. = fluorescence not detectable, i.e. quantum yield <0.001. [d] Observed fluorescence may be due to impurities.

ties examined, as listed in the Table. Fluorescence data are given for air saturated solutions and results are calculated relative to a value of 0.27 for anthracene.[8-10] (An air saturated solution of an anthracene of quantum yield

>0.2 is quenched by a factor of ca. 1.4 relative to a degassed or nitrogen-flushed solution.[8,11])

It is clear that the pyridinium or 4-methylpyridinium groups strongly quench anthracenyl fluorescence when bonded either directly or via a methylene group to the 9-position of anthracene. The N-methylimidazolium group, however, has no such quenching effect. These observations are consistent with a mechanism of quenching involving electron or charge transfer.

The electronic absorption spectra of all compounds (I) and (II) are typically anthracenic in character, and thus indicate that any ground state interaction between the carbocyclic and heterocyclic chromophores is insignificant. Thus the observed quenching must involve the interaction of excited anthracene with the quenching ligand. This behaviour clearly indicates the formation of an exciplex. Details of its structure and lifetime are, however, not clear, and depend on the physical nature of the interaction of the two chromophores. Corrected emission intensities were linearly related to absorbances up to 1.0 O.D. units cm^{-1} for all compounds investigated and thus precluded concentration-dependent quenching effects arising from intermolecular interactions.

Intermolecular exciplexes have usually been visualised in terms of a sandwich structure,[10] or a structure in which the quencher sits over the molecular plane of the fluorescer so as to give maximum overlap of orbitals. Taylor and Hammond have suggested such a structure for the naphthalene–diene exciplex,[12] and a number of intramolecular exciplexes have been described in which the quenching and fluorescing parts of the molecule are separated by a carbon chain of sufficient length to allow them to align in such a face-to-face manner.[10]

In the present cases, the limited separation of the chromophores completely precludes such an alignment. Hence for these compounds, the exciplex cannot involve a parallel plane type of structure. This same steric constraint also effectively excludes the involvement of hydrogen bond formation or proton transfer, as suggested by Ermolaev et al.[3] for the intermolecular exciplexes.

The quenching of fluorophores by intermolecular CT interaction is solvent dependent, and this dependence has been studied by several workers. Processes leading to an increase in ionic character are expected to be favoured in polar relative to apolar solvents. Mataga has given a relation connecting the ionic photodissociation yield of excited donor–acceptor systems with the solvent dielectric constant.[13] Kosower has investigated the effect of solvent polarity on the fluorescence yields of the 6-N-arylaminonaphthalene-2-sulphonate intramolecular donor–acceptor system.[14] He has shown that, whereas radiation is the major decay route in apolar solvents, electron transfer becomes competitive with

[8] A. Z. Britten and G. Lockwood, to be published.
[9] J. N. Demas and G. A. Crosby, J. Phys. Chem., 1971, 75, 991.
[10] J. B. Birks, ' Photophysics of Aromatic Molecules,' Wiley–Interscience, London, 1970.
[11] G. Heinrich, S. Schoof, and H. Gusten, J. Photochem., 1974—1975, 3, 315.

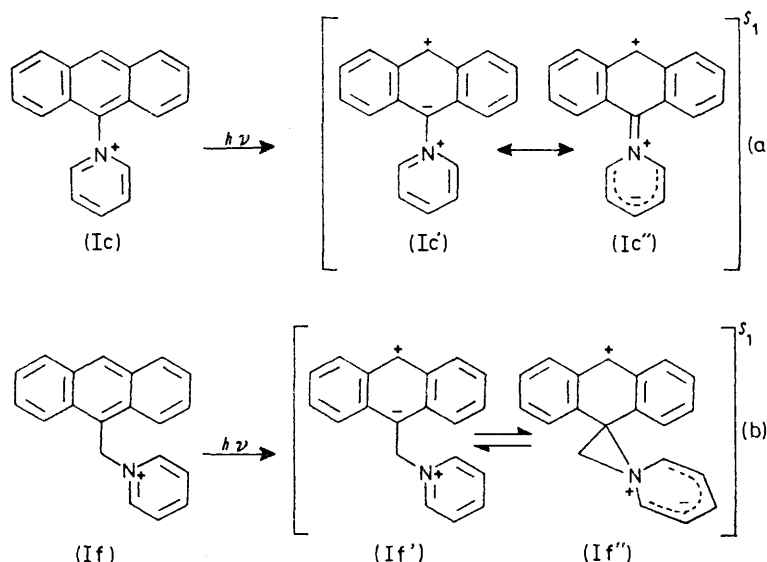[12] G. N. Taylor and G. S. Hammond, J. Amer. Chem. Soc., 1972, 94, 3687.
[13] H. Masuhara, T. Hino, and N. Mataga, J. Phys. Chem., 1975, 79, 994.
[14] E. M. Kosower, H. Dodiuk, K. Tanizawa, M. Ottolenghi, and N. Orbach, J. Amer. Chem. Soc., 1975, 97, 2167.

radiative decay in more polar solvents and the fluorescence yield drops from e.g. 0.5 in dioxan to 0.01 in water.

Electron transfer in the compounds under investigation will lead either to breakage of the bond between the chromophores, (photolysis) or to formation of some new bond, which may lead to photoproducts or may break to reform the original molecule in the ground state. In one experiment, compound (Ic) at a concentration of 0.03mM was exposed to 366 nm radiation of sufficient duration for each molecule on average to absorb, and dissipate, ca.

length, a system similar in geometry to ours, albeit of the opposite polarity. They found appreciable quenching even with direct linkage, which they attributed to charge transfer. In a similar study of some naphthylalkylamines, both α- and β-dimethylaminonaphthalenes exhibited essentially complete quenching of naphthalenyl fluorescence, for which Chandross and Thomas [17] suggested a structure of an internally bonded intermediate.

In an analogous fashion, the processes shown in the Scheme provide a mechanism for the intramolecular

SCHEME

16 photons. The absorption spectrum was unaltered at the end, indicating that no significant photochemical change had occurred. Clearly then, the exciplex structure must involve some new bond formation, and hence some steric strain in the molecule. Since the quenching is of high efficiency, the electronic stabilisation must overcome the steric destabilisation. If this change is a charge transfer process, it will lead to an increase in polarity of the molecule which must presumably be stabilised by the solvent.

The fluorescence behaviour of compounds (Ic) and (IIb and c) were similar in both ethanol and dichloromethane (dielectric constants [15] respectively 24.3 and 8.9). Unfortunately, solubility problems precluded the use of solvents with very low dielectric constants, e.g. cyclohexane and diethyl ether, in which it was hoped that, for instance, (IIb) would show some emission.

Although it has often been assumed that some tight coupling between the fluorescer and quencher is required for quenching, there is previous evidence that the geometric requirement is only weak. Okada et al.[16] have examined the 9-anthryl chromophore linked to 4-dimethylaminophenyl by a methylene bridge of 0—3 atoms

quenching by pyridinium ligands. In process (a), there is restricted rotation about the C(9)–N bond which requires the pyridine ring to lie in a plane skewed to that of the anthracene. That necessarily means that the charge-delocalised species (Ic″) must be of higher energy than the ylide (Ic′). Nevertheless, both these structures contribute to the excited state to make it manifestly non-anthracenic in character, and thereby account for the quenching of fluorescence for compounds (Ic and d).

For compound (If), and also (IIb), it is sterically feasible to develop a new covalent structure for the exciplex state involving the spiro-species (If″). This compound does not exhibit photolysis under 366 nm irradiation and moreover, it is clear that relief of strain in (If″) by rupture of the C–N bond in the three-membered ring is the most predictable process for return to the ground state (If).

Further support for this suggestion comes from the fact that neither of the imidazolium salts (Ig) and (IIc) exhibits quenching of fluorescence. For them, no

[15] F. W. Fowler, A. R. Katritzky, and R. J. D. Rutherford, J. Chem. Soc. (B), 1971, 460.

[16] T. Okada, T. Fujita, M. Kubota, S. Masaki, and N. Mataga, Chem. Phys. Letters, 1972, 14, 563.

[17] E. A. Chandross and H. T. Thomas, Chem. Phys. Letters, 1971, 9, 393.

effective charge transfer to the imidazolium ring can be envisaged.

We therefore conclude that the fluorescence of anthracene is readily quenched by proximate heterocyclic electron acceptors by a mechanism which involves electron transfer to the heterocyclic group and does not require a face-to-face interaction of the two π-electron systems.

This study establishes an important difference between the quenching capabilities of π-deficient and π-excessive heterocyclic groups bound covalently to a polycyclic hydrocarbon which has potentially valuable implications for studies on the binding of polycyclic aromatic hydrocarbons to DNA residues. By analogy with the present results, it seems reasonable to predict that covalent linkage of such hydrocarbons to N(7) or C(8) of adenine and guanine will not quench their fluorescence, whereas linkage to N(1) or N(3) would do so. Studies on the synthesis and properties of compounds designed to verify this prediction are in progress.

EXPERIMENTAL

Materials.—Anthracene was used as supplied by B.D.H. (' blue fluorescence '), and 9-methylanthracene was obtained from Koch–Light. 9,10-Dimethylanthracene was prepared by the method of Fieser *et al.*,[18] m.p. 184—185°.

N-*Anthracen-9-ylpyridinium Bromide* (Ic).—This was prepared according to the method of Barnett and Cook.[2] The nitrate salt was obtained from a solution of the bromide (0.5 g) in water (10 ml) by addition of a slight excess of 0.1M-AgNO$_3$ solution; H$_2$S was bubbled through the resulting liquid, followed by neutralisation with dilute ammonia. After centrifugation, the clear supernatant was evaporated to dryness and the residue was recrystallised from ethanol–ether, giving yellow plates of N-*anthracen-9-ylpyridinium nitrate* (0.35 g, 90%), m.p. 165—168° (Found: C, 65.9; H, 5.05; N, 8.5. C$_{19}$H$_{14}$N$_2$O$_3$,1.5H$_2$O requires C, 66.1; H, 4.95; N, 8.1%).

N-*Anthracen-9-yl-4-picolinium Bromide* (Id).—This was synthesised in a manner analogous to (Ic) using 4-picoline instead of pyridine. The product after recrystallisation from ethanol had m.p. 152—154°.

3-(*Anthracen-9-yl*)-1-*methylimidazolium Bromide* (Ie).—9,10-Dibromo-9,10-dihydroanthracene [19] (1.5 g, 4.4 mmol) was stirred with N-methylimidazole (10 ml) for 1.5 h at room temperature. The mixture was kept at room temperature a further 2 h and then was allowed to cool overnight at 5°. The white solid produced was filtered and recrystallised from acetonitrile yielding the *bromide* (0.62 g, 41%), m.p. 290—292°. No disubstituted dihydroanthracene intermediate was isolated as in the analogous reaction with pyridine [2] (Found: C, 63.6; H, 4.75; N, 8.25; Br, 23.8. C$_{18}$H$_{15}$BrN$_2$ requires C, 63.7; H, 4.45; N, 8.25; Br, 23.6%).

N-*Anthracen-9-ylmethylpyridinium Chloride* (If).—9-Chloromethylanthracene [20] (0.5 g, 2.2 mmol) was dissolved in dry pyridine (10 ml) and kept overnight at room temperature. The product was precipitated by shaking with ether

and was recrystallised from ethanol yielding yellow needles of the *chloride* (0.6 g, 82%), m.p. 184—185° (Found: C, 73.6; H, 5.85; N, 4.0. C$_{20}$H$_{16}$ClN,1.5H$_2$O requires C, 73.2; H, 5.6; N, 4.25%), τ([^{2}H$_6$]DMSO) 0.6—2.2 (14 H, m, ArH) and 2.65 (2 H, s, CH$_2$N).

3-(*Anthracen-9-ylmethyl*)-1-*methylimidazolium   Chloride* (Ig).—This was prepared from 9-chloromethylanthracene (0.5 g) and N-methylimidazole (3 ml) by the method described for (If). The *product* was recrystallised from ethanol giving yellow needles (0.4 g, 56%), m.p. 163—164° (Found: C, 71.0; H, 5.9; N, 8.4. C$_{19}$H$_{17}$ClN$_2$,0.75H$_2$O requires C, 70.8; H, 5.8; N, 8.7%), τ(CF$_3$CO$_2$H) 1.84—2.58 (12 H, m, ArH), 3.62 (2 H, s, NCH$_2$), and 6.14 (3 H, s, NCH$_3$).

9,9'-Bianthryl (Ih) was synthesised from anthrone by the method of Barnett and Matthews.[21]

10-*Methylanthracen-9-ylmethylpyridinium   Perchlorate* (IIb).—(a) 9-*Bromomethyl-10-methylanthracene.* A solution of paraformaldehyde (3.416 g, 0.113 mol) in acetic acid saturated with HBr (50 ml), was added to a solution of 9-methylanthracene (11.0 g, 0.057 mol) in acetic acid (70 ml). The mixture was stirred at 45° for 2 h. After standing overnight at room temperature, the mixture was poured into ice–water and filtered. The yellow precipitate on crystallisation from benzene gave needles of the *product* in two crystalline forms, m.p.s 152—156 and 212—216° (Found: C, 67.35; H, 4.75; Br, 28.15. Calc. for C$_{16}$H$_{13}$Br: C, 67.4; H, 4.6; Br, 28.0%), τ(CDCl$_3$) 1.68—2.60 (8 H, m, ArH), 4.48 (2 H, s, CH$_2$), and 6.94 (3 H, 2, CH$_3$).

(b) 10-*Methylanthracen-9-ylmethylpyridinium perchlorate.* The above material (0.2 g, 0.7 mmol) was dissolved in dry pyridine and kept at room temperature for 10 h. The product was precipitated by addition of benzene and recrystallised to give the deliquescent 10-*methylanthracen-9-ylmethylpyridinium bromide*, characterised as its *picrate*, m.p. 184—187° (Found: C, 63.0; H, 4.3; N, 10.9. C$_{27}$H$_{20}$N$_4$O$_7$ requires C, 63.3; H, 3.95; N, 10.9%).

The deliquescent crude bromide (0.25 g) was dissolved in anhydrous methanol (10 ml) and treated with perchloric acid (0.15 ml) for 5 min. The yellow crystalline product was filtered and recrystallised from methanol yielding needles of the *perchlorate* (0.214 g, 81%), m.p. 200—204° (Found: C, 65.9; H, 4.75; N, 3.55; Cl, 9.15. C$_{21}$H$_{18}$ClNO$_4$ requires C, 65.7; H, 4.75; N, 3.65; Cl, 9.25%).

3-(10-*Methylanthracen-9-ylmethyl*)-1-*methylimidazolium Perchlorate* (IIc).—9-Bromomethyl-10-methylanthracene (0.142 g, 0.5 mmol) in N-methylimidazole (15 ml) was heated at 50° for 2 h, and kept overnight at room temperature. Excess of N-methylimidazole was removed by extracting several times with ether and an ether-insoluble brown oil was collected. The bromide adduct did not crystallise, but was converted into the perchlorate by dissolving 0.1 g (0.27 mmol) in methanol (5 ml) and treating with perchloric acid (0.1 ml) for 5 min. The yellow crystalline residue was filtered and recrystallised from methanol giving the *product* (0.08 g, 40%), m.p. 194—197° (Found: C, 60.9; H, 5.15; N, 7.05; Cl, 9.35. C$_{20}$H$_{19}$ClN$_2$O$_4$0.5H$_2$O requires C, 60.7; H, 5.1; N, 7.1; Cl, 8.95%); τ(CD$_3$OD) 1.34—2.87 (11 H, m, ArH), 3.68 (2 H, s, Ar-CH$_2$N), 6.18 (3 H, s, NCH$_3$), and 6.90 (3 H, s, ArCH$_3$).

Fluorimetric Methods.—Fluorescence quantum yields were measured on a spectrophotofluorimeter of conventional design built in this laboratory. Light from a 450 W xenon

[18] R. B. Sandin and L. F. Fieser, *J. Amer. Chem. Soc.*, 1940, **62**, 3098.
[19] E. de B. Barnett and J. W. Cook, *J. Chem. Soc.*, 1924, 1084.
[20] F. Stewart, *Austral. J. Chem.*, 1960, **13**, 478.
[21] E. de B. Barnett and M. A. Matthews, *J. Chem. Soc.*, 1923, **123**, 380.

arc was monochromated and chopped before being focused on the sample. Emission at right angles was monochromated, before detection by an extended S20 response photomultiplier, the signal from which was fed to a phase-sensitive detector (p.s.d.) together with a reference signal from the chopper. The p.s.d. output signal was divided by a voltage derived from a Rhodamine B quantum counter which samples the incident beam *via* a quartz plate beam splitter.

A modification [8] of the method of Parker and Rees,[22] which eliminates the need for separate absorbance measurements, was used for quantum yield determinations.

[22] C. A. Parker and W. T. Rees, *Analyst*, 1960, **85**, 587.