Concerning the Fluorescence of Some 7-Hydroxycoumarins and Related Compounds

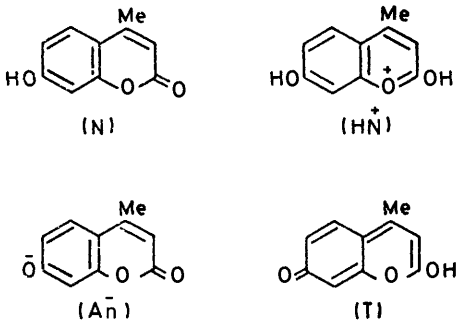
By **Godfrey S. Beddard** and **Miss Sheena Carlin,** The Davy-Faraday Laboratories, The Royal Institution, London W1X 4BS
R. Stephen Davidson,* Department of Chemistry, The University, Leicester LE1 7RH

The fluorescence of 7-hydroxy-4-methylcoumarin and 4-carboxymethyl-7-hydroxycoumarin and some simple derivatives, has been examined. For compounds having a free 7-hydroxy-group, complex fluorescence spectra were obtained when solvents containing water were employed. The complexity is in part due to the ground state equilibrium, phenol $\rightleftharpoons$ phenolate anion. This means that unless the excitation wavelength is carefully selected, more than one species is excited. Four fluorescent species, derived from the phenol, were observed in various aqueous alcohol systems: two species were identified as the excited phenol and its anion and the other two tentatively identified as an intimate and sovent separated ion-pair. From steady state fluorescence measurements and time resolved ns fluorescence spectroscopy the inter-relation of these various excited states was established. The role of water in these systems is discussed.

ALTHOUGH it is now common knowledge that coumarins such as 7-hydroxy-4-methylcoumarin can be used in tunable dye-lasers,[1,2] the nature and inter-relation of the species produced by excitation of the coumarin is the subject of much speculation.[2-6] In neutral aqueous ethanol solution, three bands are observable at 385, 450, and 485 nm.[6] respectively. By a careful increase in the acidity of the solution a further band at 520 nm becomes observable and at a sufficiently low pH, a single band at 410 nm remains.[5] If the solution is rendered basic, a single band at 450 nm is observable. The 385, 410, and 450 nm emissions have been characterised as being due to (N*), ($\overset{+}{\text{H}}$N*), and ($\overset{-}{\text{A}}$n*) which are the excited singlet states of (N), ($\overset{+}{\text{H}}$N), and ($\overset{-}{\text{A}}$n). The emissions at 485 and 520 nm, arbitrarily labelled as (X*) and (Y*), are sug-

[1] C. V. Shank, A. Dienes, A. M. Trozzolo, and J. A. Myer, *Appl. Phys. Letters*, 1970, **16**, 405.
[2] A. Dienes, C. V. Shank, and A. M. Trozzolo, *Appl. Phys. Letters*, 1970, **17**, 189.
[3] A. M. Trozzolo, A. Dienes, and C. V. Shank *J. Amer. Chem. Soc.*, 1974, **96**, 4699.
[4] G. J. Yakatan, R. J. Juneau, and S. G. Schulman, *Analyt. Chem.*, 1972, **44**, 1044.
[5] M. Nakashima, J. A. Sousa, and R. C. Clapp, *Nature*, 1972, **235**, 16.
[6] T. Kindt, E. Lippert, and W. Rapp, *Z. Naturforsch.*, 1972, **27a**, 1371.

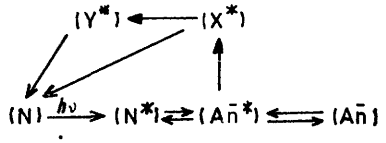
gested as being excited states derived by protonation reactions of (N*).[3-5] The most favoured structure for the ground state species which gives fluorescence at 485 nm from its excited state is tautomer (T).[3,5]



Time resolved spectroscopy has shown that (X*) is derived from (N*) and that (Y*) is derived from (X*).[2]

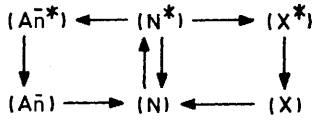
$$(N) \xrightarrow{h\nu} (N^*) \longrightarrow (X^*) \longrightarrow (Y^*)$$

Recently, from results obtained by the use of gain spectroscopy, evidence, which is thought to support the reaction sequence in Scheme 1, has been presented.[3]



SCHEME 1

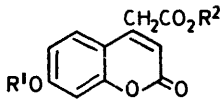
This reaction sequence has been criticised by Zinsli,[7] who from a kinetic study suggests Scheme 2. In Scheme 2 X is thought to represent the ion pair $H_3\overset{+}{O}A\overset{-}{n}$.



SCHEME 2

Despite the uncertainties surrounding the assignments of structures to the excited states of (X) and (Y), it has been established that the presence of water is an absolute necessity for formation of (X*) and (Y*).[7] Use of neutral non-aqueous solvent leads only to the formation of (N*). Upon the addition of relatively small amounts of water, solutions are obtained which show fluorescence due to (X*) (at 485 nm) and sometimes (Y*) (at 510 nm). Another prerequisite for formation of these species is that the phenolic hydroxy-group be free.[5] Compounds in which the 7-hydroxy-group is alkylated do not give the (X*) and (Y*) type emissions.

This paper presents results obtained on 7-hydroxy-4-methylcoumarin (N), its methyl ether, and compounds (A)—(D). The hydrazide of 4-carboxymethyl-7-hydroxycoumarin (A) has been proposed as an analytical



(A) $R^1 = R^2 = H$
(B) $R^1 = H$ $R^2 = Me$
(C) $R^1 = Me$ $R^2 = H$
(D) $R^1 = R^2 = Me$

reagent for carbonyl compounds on account of the fluorescent properties of hydrazones derived from it.[8] It will be seen that in (A) and (C) a free carboxy-group is present. If therefore the excited states of these coumarins are susceptible to undergoing protonation reactions, they have a suitable internal source for delivery of a proton.

EXPERIMENTAL

Compounds (A)—(D) were prepared according to literature methods.[8] Fluorescence spectra were recorded on Baird Atomic SF 100E and Perkin-Elmer MPF-4 instruments. Time-resolved fluorescent spectra were recorded on an instrument which has been previously described.[9]

RESULTS

7-Methoxy-4-methylcoumarin, (C), and (D) exhibit strong fluorescence at 385 nm in aqueous ethanol solutions. No other bands were observable. In rigid ethanol matrix (77 K) weak phosphorescence at 445 and 480 nm was also detectable.

7-Hydroxy-4-methylcoumarin (N), (A), and (B) exhibited much more complex fluorescence spectra in aqueous ethanol. The shape of the fluorescence spectra of (N) and (B) are dependent on the excitation wavelength (Figure 1). This dependence on excitation wavelength is due to the fact that at <370 nm two ground state species *i.e.* the anion and the neutral compound, are being excited. In the case of (A) no evidence could be found from its excitation spectra for the formation of its ground state anion. As can be seen from Figure 2 some anion is formed in the excited state.

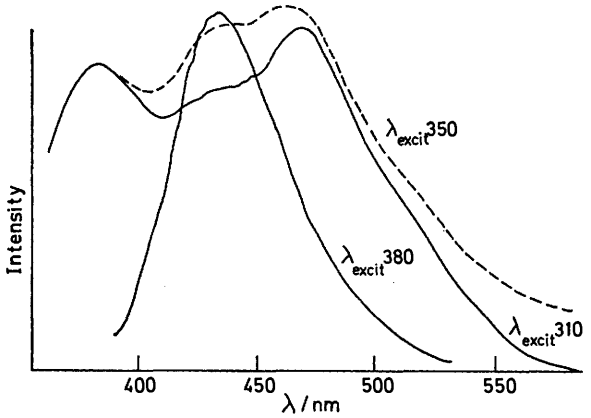


FIGURE 1 Fluorescence spectra of 7-hydroxy-4-methylcoumarin in aqueous ethanol solution

The dissociation of 7-hydroxy-4-methylcoumarin (N) to give its anion ($A\overset{-}{n}$) in ethanolic solution is dependent upon

[7] P. E. Zinsli, *J. Photochem.*, 1974, **3**, 55.
[8] J. Wilson-Baker, C. N. Haksar, and J. F. W. McOmie, *J. Chem. Soc.*, 1950, 170.
[9] G. S. Beddard, S. Carlin, and C. Lewis, *J.C.S. Faraday II*, 1975, **71**, 1894.

the amount of water present. In dry ethanol, only fluorescence characteristic of (N*) is observable. Since the amount of ($\bar{An}$) can be determined by measurement of the intensity of fluorescence from ($\bar{An}$*) when excitation wavelengths of >370 nm are employed, the dependence of the concentration of ($\bar{An}$) upon the water concentration could be easily assessed. The results are shown in Figure 3. At fairly high water concentrations, the emission spectrum of (N) is dominated by fluorescence from excited anion ($\bar{An}$*) much of which is derived by excitation of the ground state anion ($\bar{An}$). From the work of Zinsli (Figure 2 of ref. 7) it is known that

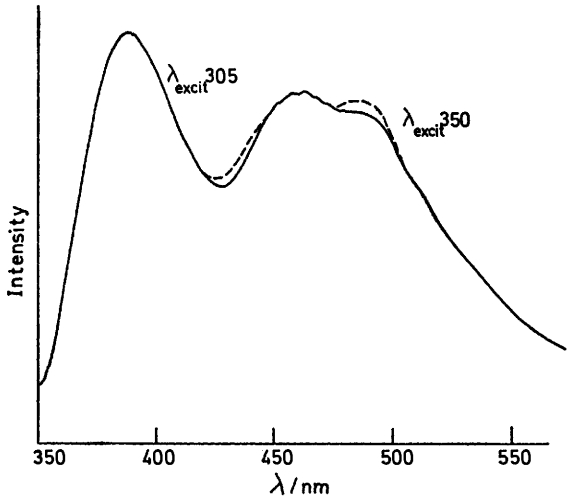


FIGURE 2 Fluorescence spectra of 4-carboxymethyl-7-hydroxycoumarin in aqueous ethanol solution

as the water concentration is increased, emission from X* predominates over the emission from ($\bar{An}$*) and then at even higher concentrations, emission from ($\bar{An}$*) predominates over that from X*. Similar results are obtained for aqueous methanol solutions.

The use of ethanolic solutions of (N) containing a very

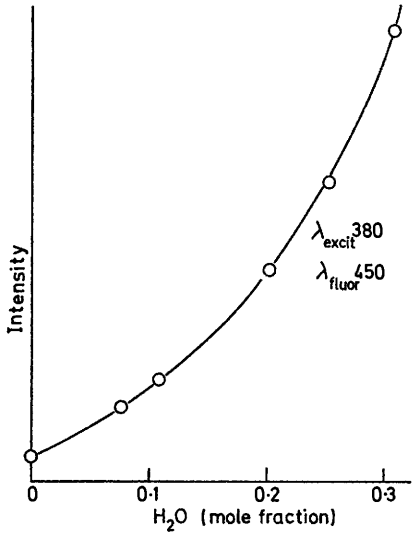


FIGURE 3 Variation in intensity of fluorescence of 7-hydroxy-4-methylcoumarin in ethanol solution containing varying amounts of water

small amount of water gives rise to reasonably strong fluorescence from (N*) and (X*). Fluorescence from (Y*) is just detectable. Such a solution was examined by the technique of time-resolved fluorescence spectroscopy, and the spectrum obtained is shown in Figure 4. It can be readily seen that (N*) is the precursor of (X*). Use of solutions containing a larger amount of water showed that most of the fluorescence from ($\bar{An}$*) occurs very soon after the exciting pulse (see Figure 5).

The dependence of the efficiency of fluorescence from ($\bar{An}$*) and (X*), derived from ($\bar{An}$) and (N) respectively, as a func-

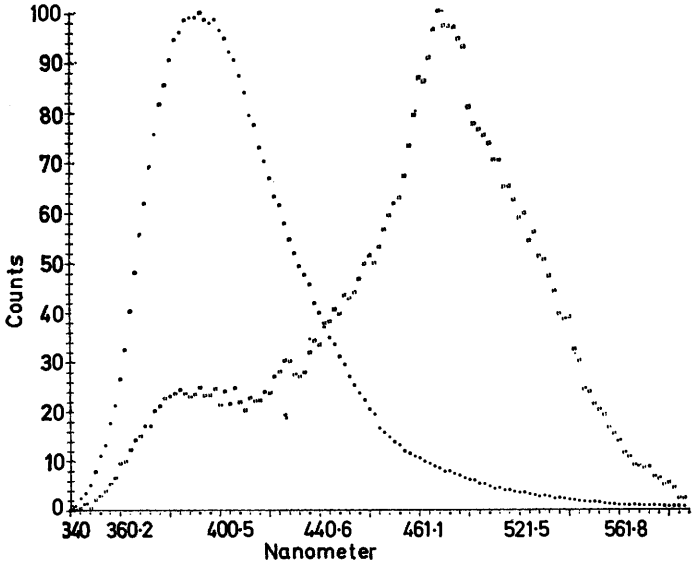


FIGURE 4 Time resolved fluorescence spectrum of 7-hydroxy-4-methylcoumarin in ethanol containing 2% water; spectrum with lower wavelength peak, 0–6 ns; Spectrum with higher wavelength peak 18–33 ns

tion of temperature, was investigated using an aqueous ethanolic solution of (N). The results are shown in Figures 6 and 7. It will be noted that fluorescence from ($A\bar{n}$*) and (X*) became unobservable at the same temperature, *ca.* —70°.

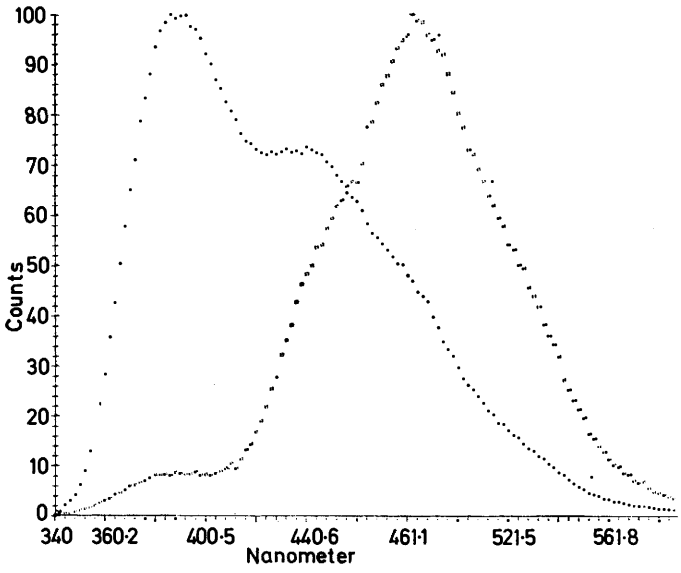


FIGURE 5 Time resolved fluorescence spectrum of 7-hydroxy-4-methylcoumarin in ethanol containing 10% water; spectrum with lower wavelength peak 0—6 ns; spectrum with higher wavelength peak, 18–33 ns

The fluorescence spectrum of (B) in t-butyl alcohol solution is particularly interesting (Figure 8) since emission from (X*) and (Y*) is also observable. A t-butyl alcohol solution of (N) exhibited a fluorescence spectrum similar to that of (B).

The room temperature emission of (N) in dry glacial acetic acid consists only of fluorescence from (N*). In contrast, use of aqueous acetic acid (50 : 50 v/v) gives rise to emission which is almost entirely fluorescence from (X*). Use of solvent hexafluoroisopropyl alcohol caused it to fluoresce only from (N*).

DISCUSSION

The finding that for compounds 7-hydroxy-4-methylcoumarin (N) and 7-hydroxy-4-methoxycarbonylmethylcoumarin (B) in aqueous ethanol solution, two ground state species are present, is of paramount importance in unravelling the inter-relation of the various ground and excited state species. Confining our attention to (N), we see that excitation of ($A\bar{n}$) produces ($A\bar{n}$*) to the exclusion of (N*), (X*), and (Y*). Since use of a different excitation wavelength gives rise to fluorescence from (N*) and (X*) we can say that (N*) and (X*) are stable in this solvent system, and therefore deduce that inequality (1) applies.

$$(A\bar{n}^*) \not\rightarrow (N^*) \text{ and } (X^*) \text{ or } (Y^*) \qquad (1)$$

In ethanolic solutions containing very little water, excitation at wavelengths <370 nm show fluorescence from (N*) and (X*). Since under these conditions, fluorescence from ($A\tilde{n}$*) is not observed it appears that conversion of either (N*) or (X*) into ($A\bar{n}$*) is inefficient. By means of time-resolved spectroscopy it was possible to show that in these solutions,(N*) is the precursor of (X*) and (Y*). The fluorescence spectrum of the solution, after a short time interval has elapsed since excitation, exhibits fluorescence only from (N*) (see Figure 4). Examination after a longer time interval shows fluorescence from both (X*) and (Y*). There is a hint that some ($A\tilde{n}$*) may be present and this suggests that (X*) or (Y*) may act as a precursor of ($A\bar{n}$*). That (Y*) is derived from (X*) has been previously demonstrated. The time-resolved spectra of ethanolic solutions containing a larger amount of water (see Figure 5) show that ($A\bar{n}$*) and (N*) are produced in the excitation process. The fluorescence spectrum 18—33 ns after excitation shows the presence of both (X*) and ($A\bar{n}$*) and a trace of (Y*). It is hard to determine the origin of ($A\bar{n}$*) from this

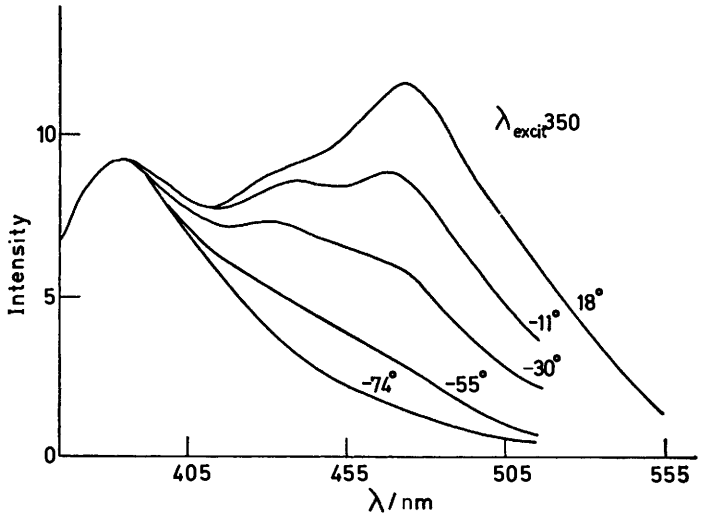


FIGURE 6 Changes in the fluorescence spectrum of 7-hydroxy-4-methylcoumarin in aqueous ethanol, caused by varying the temperature of the solution ($\lambda_{exc.}$ 350 nm)

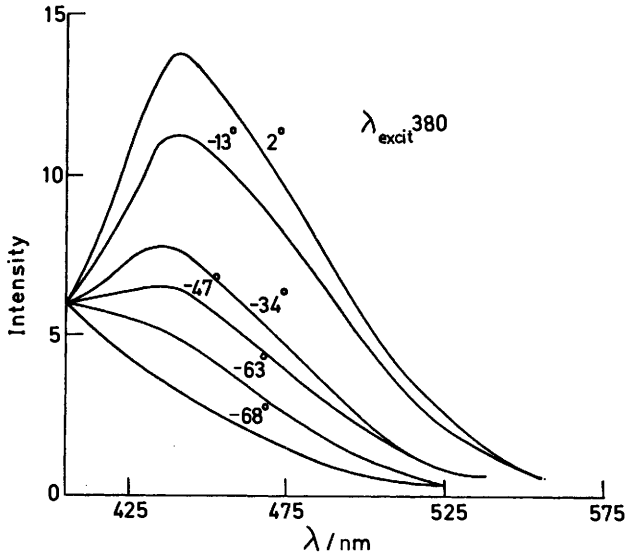


FIGURE 7 Changes in the fluorescence spectrum of 7-hydroxy-4-methylcoumarin in aqueous ethanol, caused by varying the temperature of the solution ($\lambda_{excit.}$ 380 nm)

spectrum. From these experiments using aqueous ethanol we can tentatively draw up Scheme 3.

The experiments using 4-carboxymethyl-7-hydroxycoumarin (A) are particularly illuminating. In dry methanol and ethanol solutions, the intensity of emission at 485 nm is comparable to that obtained by excitation of compound (B) under similar conditions. If formation of species which gives rise to emission at 485 nm involves a prototropic process emission at this wavelength should have been much more intense for (A) since this compound contains a proton source. This, together with the fact that the fluorescence of 7-hydroxy-4-methylcoumarin (N) in glacial acetic acid is solely due to (N*) argues against the species which fluoresce at 485 nm being produced by a process involving protonation of the coumarin system.

A further interesting feature of (A) is that it does not give rise to detectable quantities of its anion in aqueous ethanol solution. This must be due to the carboxy-group acting as a local proton source and so either suppressing ionisation or protonating the anion soon after its formation. It seems unlikely that the carboxy-group deactivates the phenolic hydroxy-group by its inductive effect since ground state anion formation in aqueous ethanol occurs with 7-hydroxy-4-methoxycarbonylmethylcoumarin (B). Although ground state anion formation from (A) could not be detected in aqueous ethanol solution, formation of the excited anion could be easily seen. This species must be formed either directly from the excited singlet state of (A) or from the species which gives rise to the emission at 485 nm. Unfortunately deductions cannot be made about the acid–base equilibria of the excited singlet states of (N) and (B) in aqueous ethanol solutions due to the large amount of ground state anions in these systems. Any choice of excitation wavelength leads to some of the ground state anion being excited. However, recently described experiments [3] suggest that (N*) ⟶ (An⁻*) occurs in slightly acidic medium.

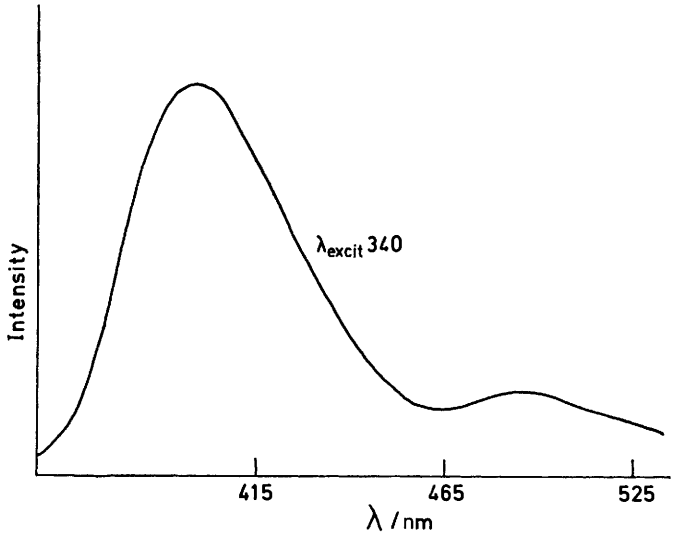
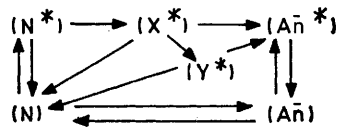


FIGURE 8 Fluorescence spectrum of 7-hydroxy-4-methylcoumarin in t-butyl alcohol solution

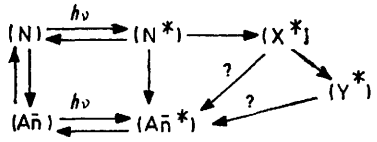


SCHEME 3

These experiments establish that ground and excited state acid–base equilibria of phenols in alcoholic solution are profoundly affected by the amount of water present in the solvent. That the ground state equilibria of phenols with their anions in aqueous methanol solution is affected by the water content has been the subject of a recent quantitative study.[10] This work showed that

[10] G. H. Parsons and C. H. Rochester, *J.C.S. Faraday I*, 1975, 1058, 1069.

there is a very marked rise in the pK_a values of phenols as the water content decreases from *ca.* 10 to 0%. One may also anticipate that excited state pK_a values will increase as the water content of alcoholic solutions decreases. Presumably there is a minimum number of water molecules required to surround an excited phenol molecule before dissociation can occur. This will produce an intimate ion pair and to obtain solvent separated ions further water molecules will need to be present. If



SCHEME 4

the water content is low, the excited singlet state of the neutral phenol will be able to undergo radiative decay before the requisite number of water molecules are present to enable dissociation to take place.

The inter-relations between the various ground and excited state species derived from (N) can be pictured as occurring *via* the processes shown in Scheme 4. The question remains as to what (X*) and (Y*) are. It has been previously suggested that (X*) is the excited state of the tautomer (T) and that this is derived by reactions involving protonation of the carbonyl oxygen atom. The observation of both (X*) and Y(*) in neutral t-butyl alcohol solution and the lack of formation of (X*) and (Y*) in dry acetic acid solution strongly suggest that proton transfer to the coumarin system is not involved in the formation of (X*) and (Y*). It is the polarity of the medium that appears to be of paramount importance. The experiments with 7-methoxy-4-methylcoumarin, 4-carboxymethyl-7-methoxycoumarin (C), and 7-methoxy-4-methoxycarbonylmethylcoumarin (D) show that a free 7-hydroxy-group is required for observation of long wavelength emissions such as those exhibited by (X*) and (Y*). The finding that formation of (X*), (Ān*), and (Ān) show the same temperature dependence strongly suggests that they are formed by similar processes. We suggest that (X*) and (Y*) are the excited states of the intimate and solvent separated ion pairs derived from (N). Zinsli has previously suggested that (X*) is due to fluorescence from an ion pair. Addition of water to ethanolic solutions increases the polarity of the medium and aids the process which leads to formation of solvated ions. Addition of acid suppresses dissociation into solvent separated ions, *i.e.* retards processes (N) ⟶ (Ān), (N*) ⟶ (Añ)*, (X*) ⟶ (Ān*), and (Y*) ⟶ (Ān*) and consequently leads to efficient production of (X*) and (Y*).

If (X*) and (Y*) are intimate and solvent separated ion pairs one may ask why the energies of the transitions (X*) ⟶ (X) (58.9 kcal mol^{-1}) and (Y*) ⟶ (Y) (56.1 kcal mol^{-1}) are lower in energy than the (Ān*) ⟶ (Ān) (63.6 kcal mol^{-1}) transition. The answer probably lies in the fact that (X) and (Y) each have an energy content greater than (An). (see Figure 9) The fact that (Ān*) does not act as a precursor of (N*), (X*), and (Y*) suggests that it has a lower energy than these species.

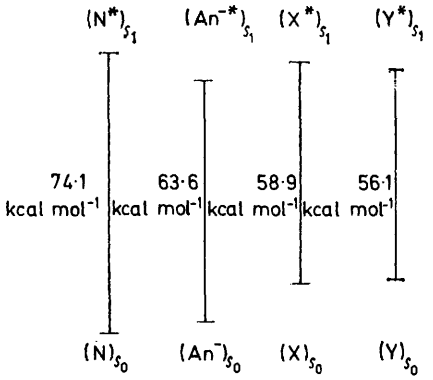


FIGURE 9 Schematic representation of the energetic relationships between (N), (Añ), (N*), (Añ*), and (X*)

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