STUDIES ON THE PHOTOLUMINESCENCE OF INDOLES

BY

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SUMMARY

The literature on the fluorescence of indole, and its application in some recent studies on the intrinsic fluorescence of proteins, is briefly reviewed. The electronic energy levels of the indole chromophore are described, with a method for analysing the overlapping ^1L_a and ^1L_b transitions, using the differential shifting of these bands caused by methyl substitution. Solvent effects on indole emission are investigated. Non - hydroxylic solvents are found to shift the spectrum in a manner explicable by polarisation interaction. Hydroxylic solvents interact more strongly with the ^1L_a state than with the ^1L_b state, causing reversal of these levels in fluorescence from indole or skatole, but not from 5 - methylindole. All these indoles form exciplexes with hydroxylic solvents, and the possible structure of the exciplexes is discussed. The interaction of the indole chromophore with the carbonyl group is investigated, and to facilitate this, several indoles were synthesised using the Fischer indole reaction. The ketonic carbonyl quenches by excitation transfer fron indole to the ketone, whilst the non - ketonic carbonyl forms a donor - acceptor complex with the excited indole. Quenching is dependent on the electron affinity of the non - ketonic carbonyl group. These studies are compared with reported studies on related benzene derivatives. Comments are made on the determination of quantum yields and lifetimes.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. A. Z. Britten for his help and encouragement throughout the course of this work.

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CONTENTS

INTRODUCTION

Early work on the fluorescence of proteins, around 1955 - 1957 [1], showed that the emission occurred from only the aromatic amino acid residues. With excitation of 260 - 290nm, only tryptophan, tyrosine and phenylalanine absorb. Any contribution to the emission by phenylalanine, with the lowest extinction coefficient and a quantum yield of 0.04, was shown to be negligible. Tyrosine and tryptophan both were found to have quantum yields of about 0.2 [2]. Tyrosine emission proved to be extremely sensitive to the state of ionisation of the phenolic -OH group[3,4]; formation of the anion reduced the quantum yield to 0.03 [5]. By irradiating at wavelengths greater than 290nm, it was found possible to produce emission selectively from tryptophan residues, as the absorbance of tyrosine is negligible at this wavelength, and emission from tyrosinate (which absorbs at 290nm) is low. Furthermore, energy transfer from tyrosinate to tryptophan, leading to an apparently higher yield from tryptophan, was shown not to occur; the reverse was the predicted direction of transfer (i.e. quenching of tryptophan by tyrosinate) at the singlet level[6].

It was shown that by careful choice of excitation wavelength, it was possible to examine fluorescence emission from tryptophan alone. The characteristics of this emission were expected to be a good probe for the microenvironment of tryptophan in a protein molecule, particularly if the protein

were to contain only one tryptophan residue per molecule. A large number of observations of the behaviour of the luminescence of tryptophan in different environments was made during the 1960s; these have been discussed at some length by Weinryb and Steiner[7], Longworth[1], and Cowgill[3,8]. Their conclusions are basically:-

a) An emission wavelength λ_{max}= 350nm is characteristic of tryptophan in a polar environment, i.e. "fully exposed" to the solvent. This shifts to ca. 310nm in a very non - polar environment.

b) Free tryptophan (zwitterion) has a fluorescence quantum yield of 0.2; this is quenched by peptide bond formation or by esterification, but only in a polar environment. Buried residues are thus expected to have higher yields than exposed residues.

These results have been much used in protein biochemistry in recent years, in relation to protein conformation studies. Some of the more recent cases will now be mentioned.

i) Nieto et. al.[9] have found that penicillin quenched the tryptophanyl fluorescence of Streptomyces R61 DD Carboxy - peptidase transpeptidase. The unquenched emission maximum was at 320nm, but it is not clear if any wavelength shift occurred on quenching. The quenching effect took 10min for full effect, implying either a slow chemical reaction (this was discounted because of the ready reversibility of the quenching), or a conformational change. This conformational

change was interpreted as evidence for an allosteric inactivation of the enzyme by the penicillin, rather than substrate competition. Other binding studies using guanidinium chloride partially unfolded protein solutions supported this hypothesis.

ii) Ferricytochrome c has a single tryptophan per molecule. Tsong[10] found that its fluorescence was quenched almost completely by the haem group, but that unfolding in 9M urea or 7M guanidinium chloride gave a yield 60% that of free tryptophan. In 4M guanidinium chloride, other criteria suggest that the molecule is completely unfolded. However, its fluorescence is not at a maximum. This was interpreted as evidence that an unfolded protein is not in a true random coil form; it possesses residual structure which may have significance in the nucleation of chain folding processes.

iii) Pownall and Smith investigated the quenching of micellar anthracene by iodide and pyridinium ions[11], whose quenching effects were related to the net charge in the fluorophore environment. They applied this to tryptophan containing apolipoprotein - alanine and H.S.A.[12], studying the interaction of phosphatidylcholine with these proteins. They found that the quenching constants for iodide and pyridinium ions on the proteins (compared with free tryptophan) implied that the tryptophans in both proteins were in a relatively negative region, but that lipid interaction was in a positive region for the lipoprotein, no significant interaction with H.S.A. occurring.

iv) The blue copper protein azurin also contains only one tryptophan per molecule[40]. This was found by Finazzi - Agro et. al.[13,14] to emit maximally at 308nm in both the apo - and holo - protein, shifting to 345nm on unfolding by 6M guanidinium chloride or by dropping the pH to 1.5. This was interpreted as evidence for a highly hydrophobic environment of the tryptophan (consistent with absorption spectral evidence). Addition of Cu^{2+} to the apo - protein reduced the fluorescence quantum yield by a factor of 3, as did addition of Hg^{2+}. However, addition of Ag^{2+} reduced the quantum yield by only 20 - 25%. This was initially interpreted as a heavy metal quenching effect[14] (i.e. an increase of the intersystem crossing rate), but phosphorescence measurements showed identical behaviour[13] - addition of heavy metal ion quenched the phosphorescence parallel with the fluorescence. This indicated to the authors that the metals were influencing internal conversion in some way. The absence of any shift in wavelength on addition of Cu^{2+} to the apo - protein seems to preclude any conformational change (especially with such a low emission wavelength maximum), nevertheless a model was proposed in which a small conformational change occurred on coordination (depending on the coordination numbers - 4 for Cu^{2+} and Hg^{2+}, 2 for Ag^{2+}), altering the strain on the tryptophan, and hence its internal conversion rate.

v) Nieuwenhuizen et. al.[15] investigated the tryptic

activation of prephospholipase A_2, another monotryptophanyl protein. On activation, the tryptophan fluorescence shifts from 350 to 340nm and is quenched by a factor of 2.3, evidence which was taken to imply that a "probably small conformational change takes place, moving the tryptophan to a more apolar environment". Other evidence did not support the occurrence of any conformational change. The authors also determined the constants K_m and k_{cat} (where $V_{max} = k_{cat}/[E]_o$, using standard enzymological symbolism) for the activation reaction, but their figures ($K_m = 0.041mM$, $k_{cat} = 0.36s^{-1}$) wildly disagreed with earlier measurements from enzyme activity studies[16] ($K_m = 2.2mM$, $k_{cat} = 7s^{-1}$). No clear reasons for these dis - crepancies were given.

These five examples illustrate that fluorescence measurements are being used as evidence in the postulation of new theories or as evidence against previous conclusions concerning protein structure. The presently inexplicable anomalousness of some of the results would, however, imply that such use may be misleading. It is insufficient to base conclusions such as these on a few empirical results; it is necessary to know, for instance, not only that an increase in polarity of the environment apparently red - shifts the fluorescence spectrum of tryptophan, but why this is so, - what the molecular mechanism is. As will be shown below, whilst there has been much work done on this fundamental aspect, there is also much

disagreement in the literature, which must be resolved before fluorescence measurements can be used conclusively in the study of protein structure or protein - ligand interactions.

It is the intention of this work to examine some of the fundamental problems hinted at above, and two approaches will be adopted:

a) The electronic structure and transitions of the indole chromophore itself, and their dependence on the solvent environment, will be examined;

b) The effects of proximal groups, especially those peculiar to proteinaceous tryptophan, on the indole chromophore, will be examined.

The absorption spectrum of indole around 280nm consists of two overlapping bands[17], designated ^1L_a and ^1L_b in the Platt nomenclature[18]. These have been analysed by Weber[19,20] on the basis of excitation polarisation spectra, and later by Strickland et. al. using solvent perturbation techniques[21], and substitution preturbation[22,26]. Polarised absorption spectra were analysed by Yamamoto and Tanaka[23]. The relative contributions of the two components, expressed as oscillator strengths, were found by these methods to be ca.0.02 (^1L_b) and 0.13 (^1L_a). Molecular orbital calculations gave agreement to an order of magnitude (^1L_b 0.04, ^1L_a 0.21 [24]; ^1L_b 0.08, ^1L_a 0.12 [25]).

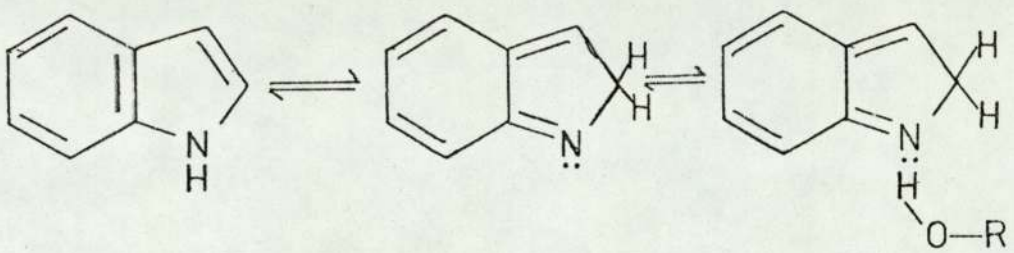
Absorption to two levels implies that emission from two

levels may be a possibility. Examination of emission polarisation spectra led Kurtin and Song[27] to conclude that emission from both ^1L_a and ^1L_b levels occurred in polar solvents, in agreement with predictions by Konev[28]. De Lauder and Wahl[29], however, found emission from only one level, the ^1L_b state. In non - polar solvents, indole emission has been fitted to a single exponential decay by De Lauder and Wahl[29], and recently by Andrews and Forster[30], who explained this by simultaneous emission from thermally equilibrated ^1L_a and ^1L_b levels, with ^1L_b - ^1A dominating the indole emission.

The emission red shift in polar solvents has been attributed by Mataga et. al.[31] to dipole - dipole interaction between solvent and solute in the excited state, leading to stabilisation of the ^1L_a state over the ^1L_b state, and hence to emission from the ^1L_a state in polar solvents. Application of Mataga's formula for orientation polarisation interaction with an excited state dipole gave a poor correlation for indole[32], and Walker et. al. observed also that concentrations of alcohols (in cyclohexane solution) too small to affect the bulk dielectric properties, gave a large red shift in the emission of indole. They suggested that exciplexes were formed between indole and solvent. This view was contested by Eisinger and Navon[33], who preferred reorientation of the solvent shell around the indole to specific exciplex formation. Whereas Walker et. al.[32] obtained 1:2 exciplex stoicheiometry,

Longworth[1] obtained only 1:1 complexes, but agreed with the exciplex theory.

There appears to be little suggestion in the literature as to the physical structure of the proposed exciplex. Chopin and Wharton[34] suggest without evidence that indole in the excited state isomerises to the 2H form, which then forms a hydrogen bond with the hydroxylic solvent:-



Consideration of the resonance energy lost in this process would suggest that a 3H indole scheme would be more likely, and indeed, Chopin[35] later modified the above in favour of an excited 3H indole. Vander Donckt[36], on the other hand, found from pH titrations that the basicity of indole is sufficiently increased in the S_1 state to allow hydrogen bond formation between the carbon atoms of the ring and the hydroxylic solvent.

The aforementioned observations will later be discussed, with further evidence relating to the structure and properties of the exciplex.

The second approach concerns the interaction of the excited state of indole with ligands other than the solvent. In particular, the side chain present in tryptophan, and proximal ligands (other amino acid side chains) must be considered.

Cowgill[3] has shown that the species in the tryptophanyl side chain responsible for the quenching of the fluorescence are the unionised carboxyl group and the ionised amino group. By examining the luminescence of several tryptophan derivatives, including esters, amides and dipeptides, he concluded that quenching was caused by the presence of a carbonyl group in a polar solvent. Thus, tryptophan ethyl ester was quenched in polar solvents, but the emission intensity rose to that of the control in apolar solvents. Van Duuren's earlier observation[37] that 3-indoleacetic acid was nonfluorescent in cyclohexane, but fluorescent in ethanol appears to be in contradiction, but observations presented here show that indoleacetic acid is, in fact, fluorescent in cyclohexane.

Various mechanisms are conceivable for the quenching process. Steiner and Kirby[38] visualised quenchers as electron scavengers, and that quenching of indole involves electron ejection from the excited indole. Weinryb and Steiner[7] mentioned the potent quenching action of the hydrogen ion as evidence for an electron transfer mechanism. This type of mechanism must involve an interaction at collisional distances between the

fluorophore and quencher, i.e. quenching is not via a dipole - dipole resonance transfer mechanism. Cowgill[8] suggested that peptide quenching originated from the intramolecular enhancement of vibrational coupling between the ground and excited states. Feitelson[39] also concluded, on the basis of a study of several indole derivatives, that collision of the quencher with the excited indole ring is necessary for the occurrence of fluorescence quenching. He further noted that quenching ability is enhanced by neighbouring polar groups, suggesting an electrostatic interaction between the excited indole ring and quencher, leading to an excited state charge transfer complex.

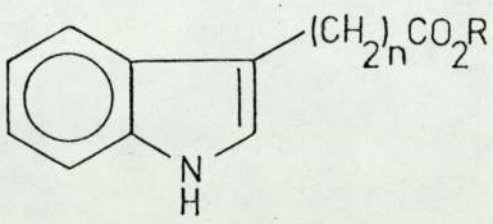
The above observations indicate that a steric requirement will be necessary for quenching, i.e. the carbonyl or other quenching group must be able to approach the indole nucleus within collisional distances. Accordingly, it was decided to synthesise several indoles containing carbonyl groups held at various distances from the indole nucleus, and rigidly held where possible. This synthetic work occupied two thirds of the total duration of this study, and is described in the following section.

SYNTHESIS OF SOME INDOLE DERIVATIVES

Introduction.

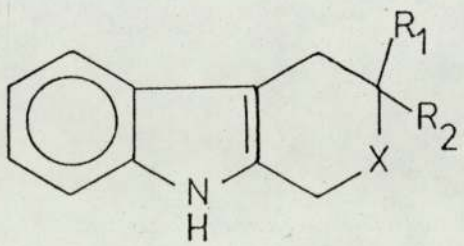
Cowgill's hypothesis [8] suggests that the quenching of fluorescence of the indole group in tryptophan and proteins is determined by the presence of the side chain carbonyl group in its particular environment. Since this carbonyl group must interact with the indole nucleus in some manner, a steric requirement is expected. Consequently, a number of indoles, each containing a carbonyl group, together with similar non - carbonyl control compounds, were synthesised. An attempt was made to synthesise compounds in which the carbonyl group was held rigidly if possible, at varying distances from the indole nucleus. To test the generality of Cowgill's hypothesis, a number of carbonyl types (ketonic, acid, amide, ester etc.) were required. Accordingly, three series of compounds were made:-

(a)

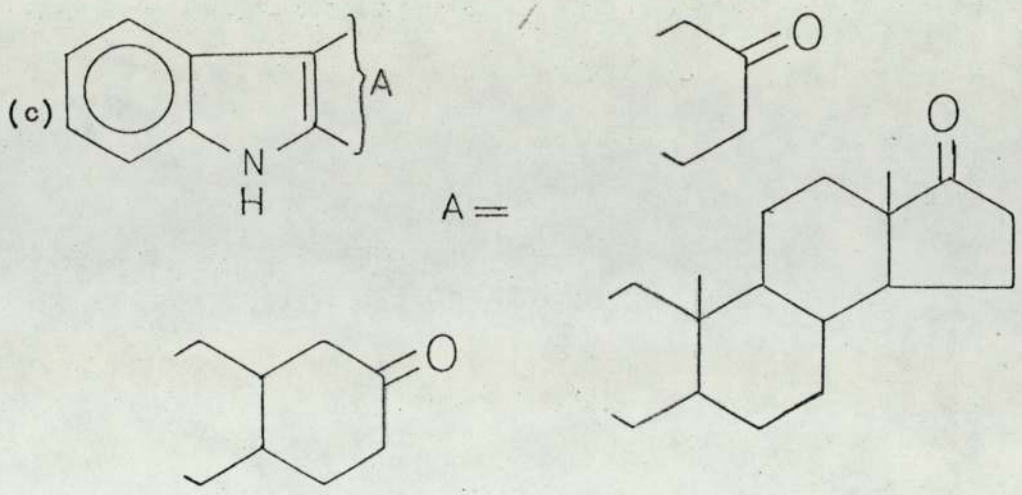


R = H, Me, Et.

(b)



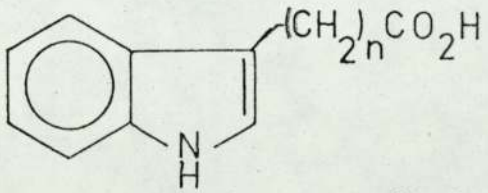
R_1	R_2	X
-COOH	-H	$=CH_2$
-COOEt/Me	-H	$=CH_2$
-COOH	$-NH_2$	$=CH_2$
-COOH	-NHAc	$=CH_2$
-COOH	-H	=NH



Also, a number of related derivatives of the above compounds, which were previously known and easily synthesised, were made. Control compounds were mainly indolyl hydrocarbons with the skeletons of the above, but included also some hydroxylic derivatives.

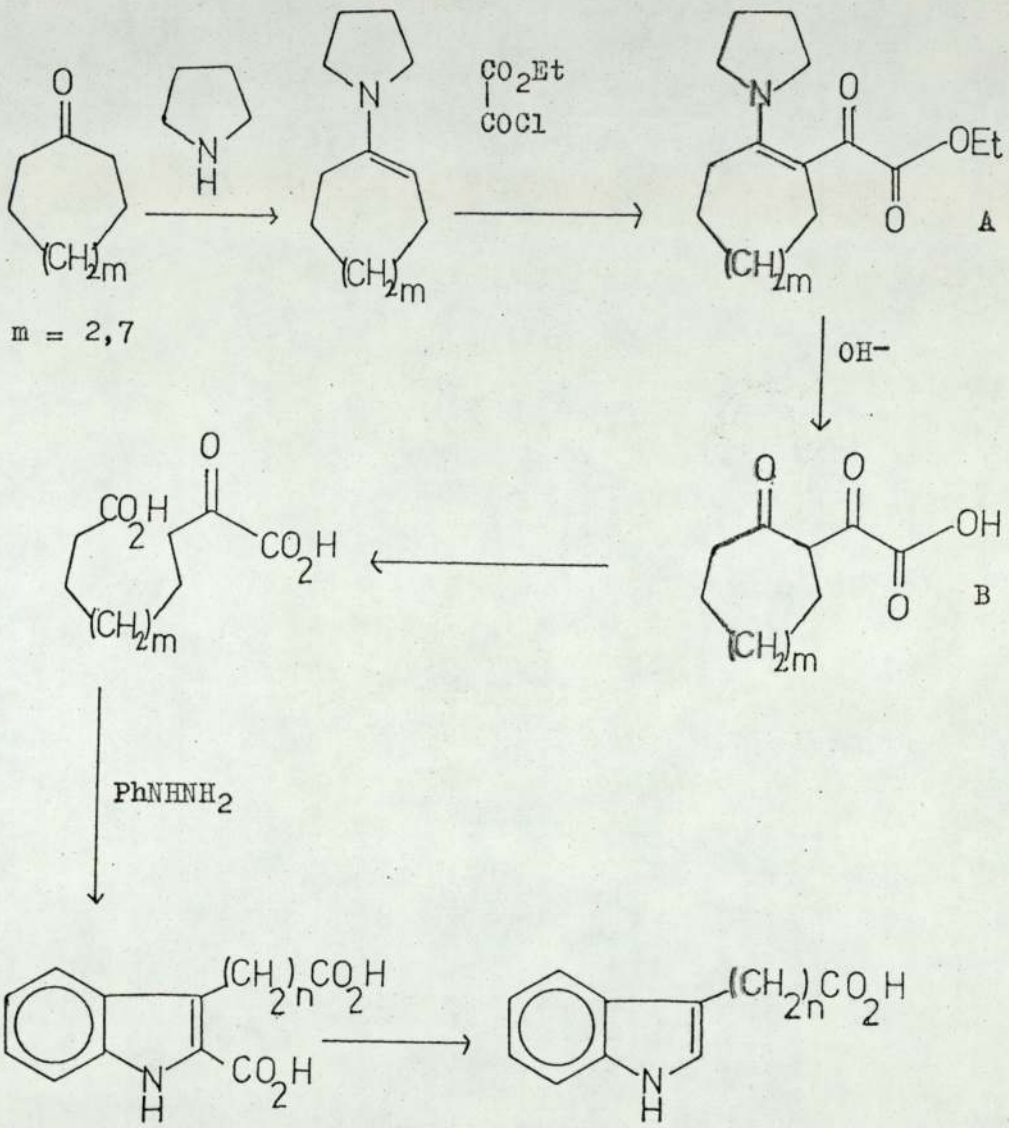
The syntheses will be considered in the group order as shown above.

(a) 3 - Indolyl - ω - Alkanoic Acids



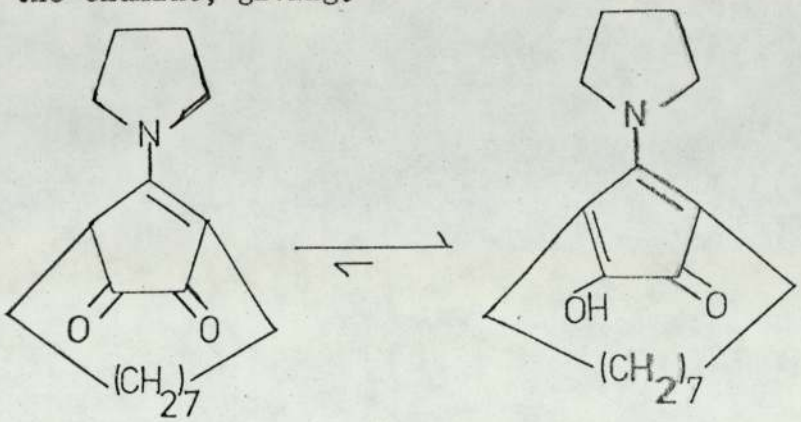
The lower members (n = 1,2,3) of this series are commercially available (in horticultural or reagent grade); these were purchased and purified by recrystallisation from water. The members n = 4,5 have been described in the literature,[41] and were not utilised in this study. An attempt to prepare indolylpentanoic acid by Kolbe electrolysis[42] using indolyl -

propanoic acid and succinic anhydride failed. Also, the following scheme was used for n = 5 and n = 10, similarly without success:-



Enamines were prepared as described by Stork et. al.[43] It was intended to ring - open the β - diketone B by alkali as described by C.R. Hauser et. al.,[44] but in the cycloheptanone case, no β- diketone was isolated, and in the cyclododecanone case, a bright yellow crystalline solid was obtained which was not fully characterised, but was possibly formed by reaction

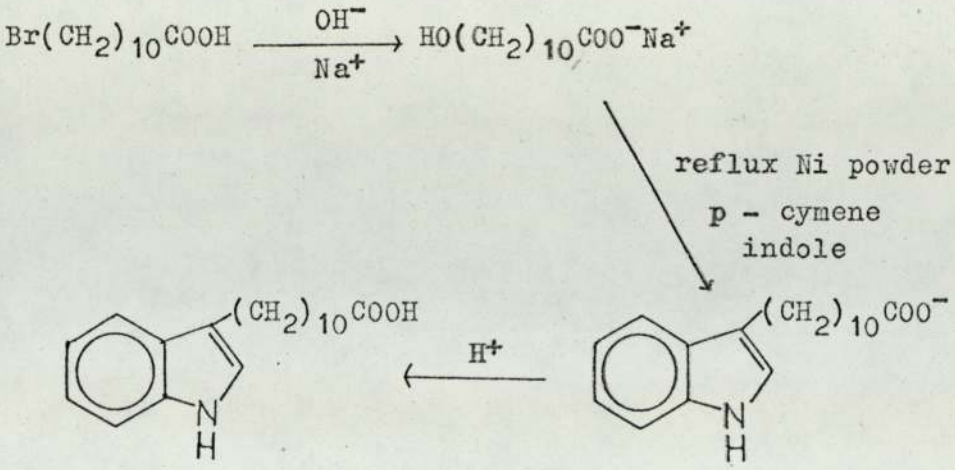
of the ester group of A with the second reactive α position of the enamine, giving:-



n = 8. Several attempts were made to synthesise this compound, starting with 11 - bromoundecanoic acid. Earlier attempts involved hydrolysis to the alcohol, followed by oxidation to the aldehydo - acid. However, it was found hard to stop the oxidation at this stage. Reagents tried included phosphoric acid + DMSO,[45] chromium trioxide / acetone, and oxidation via the tosylate[46].

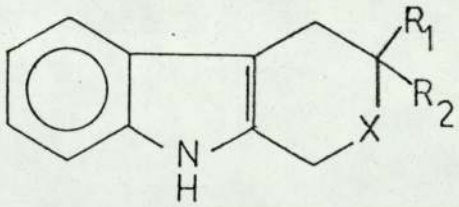
n = 9. Ethyl 11 - cyanoundecanoate was obtained from the ethyl ester of the aforementioned bromo compound, with KCN. A Stephen reaction[47] was unsuccessfully attempted on this compound, and the aldehyde required for Fischer indolisation was not obtained.

n = 10. After the failure of the enamine method, the following sequence was used, giving a low yield of a product whose spectra were consistent with those expected of indolyl - undecanoic acid:-



The sodium salt of 11 - hydroxyundecanoic acid was reacted with indole in refluxing p - cymene with nickel powder (deactivated Raney nickel) catalyst[48] . 5.9 g of sodium salt gave only about 20 mg of purified product, which easily oxidised on keeping.

(b) Tetrahydrocarbazole Derivatives



(i) X = NH

These 1,2,3,4 - tetrahydro - β - carboline derivatives were prepared by cyclising the corresponding tryptophan derivative with formaldehyde. (Acetaldehyde was used in some cases, leading to 1 - methyl derivatives). The method used was a modification of that of Jacobs and Craig[49] .

(ii) $X = CH_2$

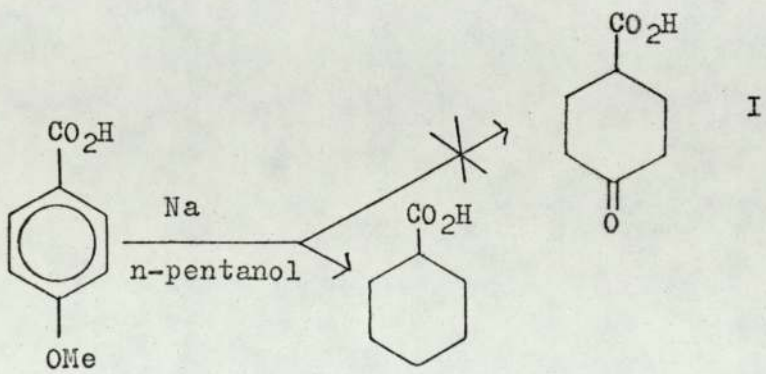
A rigid tryptophan analogue can be formed by cyclisation from the indole ring 3 position to either the 2 or the 4 position. It was decided to pursue only one of these, namely the 2,3 cyclised system, for the following reasons:

a) the 3,4 cyclised indoles are not readily accessible; only the lysergide compounds are well documented,[50] and

b) the 2,3 cyclised compounds can be prepared by a simple Fischer indole synthesis, using unsubstituted phenylhydrazine with a suitable cyclohexanone derivative.

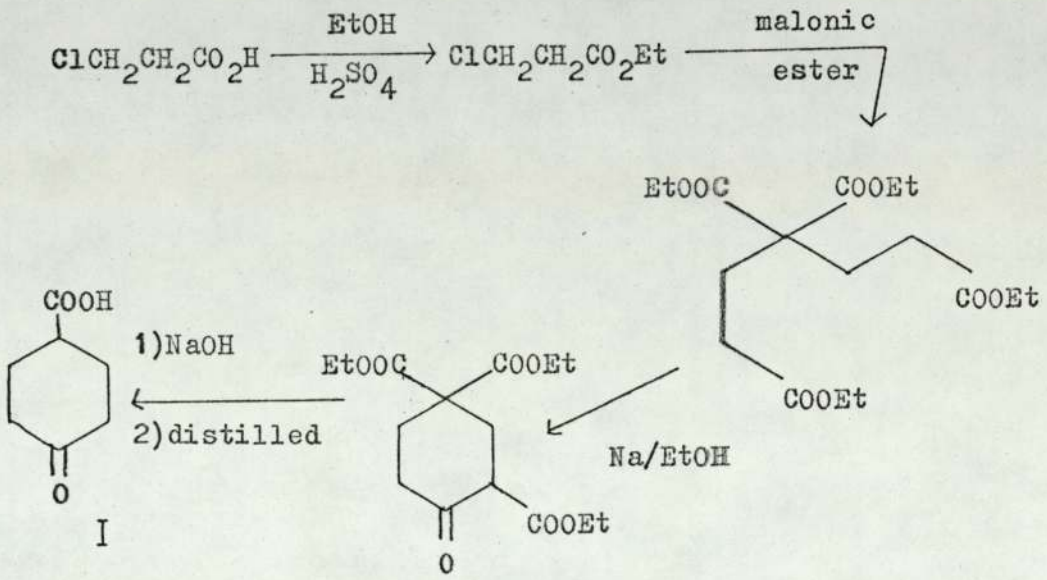
The problems associated with this series concerned the synthesis of the ketone for the Fischer indole synthesis, which in general proceeded smoothly and without complication. A mineral acid catalyst (HCl or H_2SO_4) usually gave higher yields and a cleaner reaction than the often used acetic acid (possibly because of the greater ease of workup in the ether extraction stages).

Several attempts were made to prepare cyclohexanone derivatives by reduction of suitable benzenes, e.g. anisic acid, p - hydroxybenzaldehyde, methyl p - hydroxybenzoate. These either failed, or gave products in which the substituent was also reduced off, e.g.[51,52,53]



I

The above keto acid (I) was eventually prepared by the following route, as described by Hardegger and Plattner:[54]

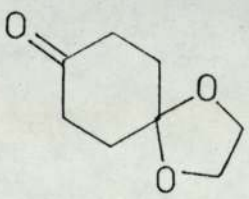


A one hour reflux of compound I with phenylhydrazine in glacial acetic acid gave a high yield of the required indole - acid; using conc. H_2SO_4 in ethanol, a high yield of the ethyl ester of 1,2,3,4 - tetrahydrocarbazole - 3 - carboxylic acid was obtained.

The amino acid derivatives proved harder to obtain. From quinitol (cyclohexane - 1,4 - diol), 4 - oxocyclohexyl benzoate was made, by the method of Jones and Sondheimer.[55] A Strecker reaction on this gave the desired aminonitrile, but attempts to hydrolyse the benzoate and/or nitrile led to mixtures of products. Protection of the amino group with the benzyloxycarbonyl (BOC) group (by reaction with benzyl chloroformate[56]) gave a stable compound which was resistant

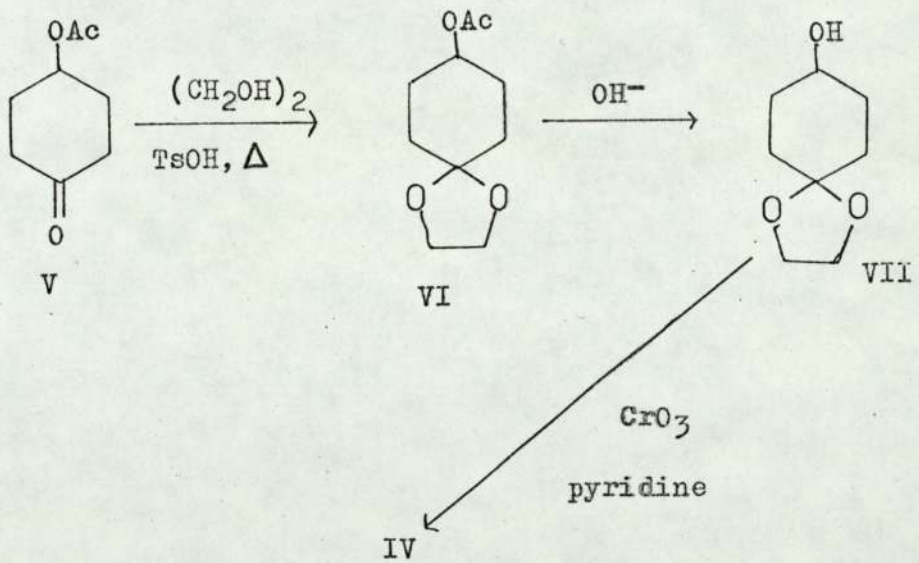
to hydrolysis. Attempts to oxidise the products of hydrolysis of the initial aminonitrile would not yield any ketone.

Finally, the desired product was obtained from the half protected dione IV. This was obtained from 4 - acetoxy -



IV

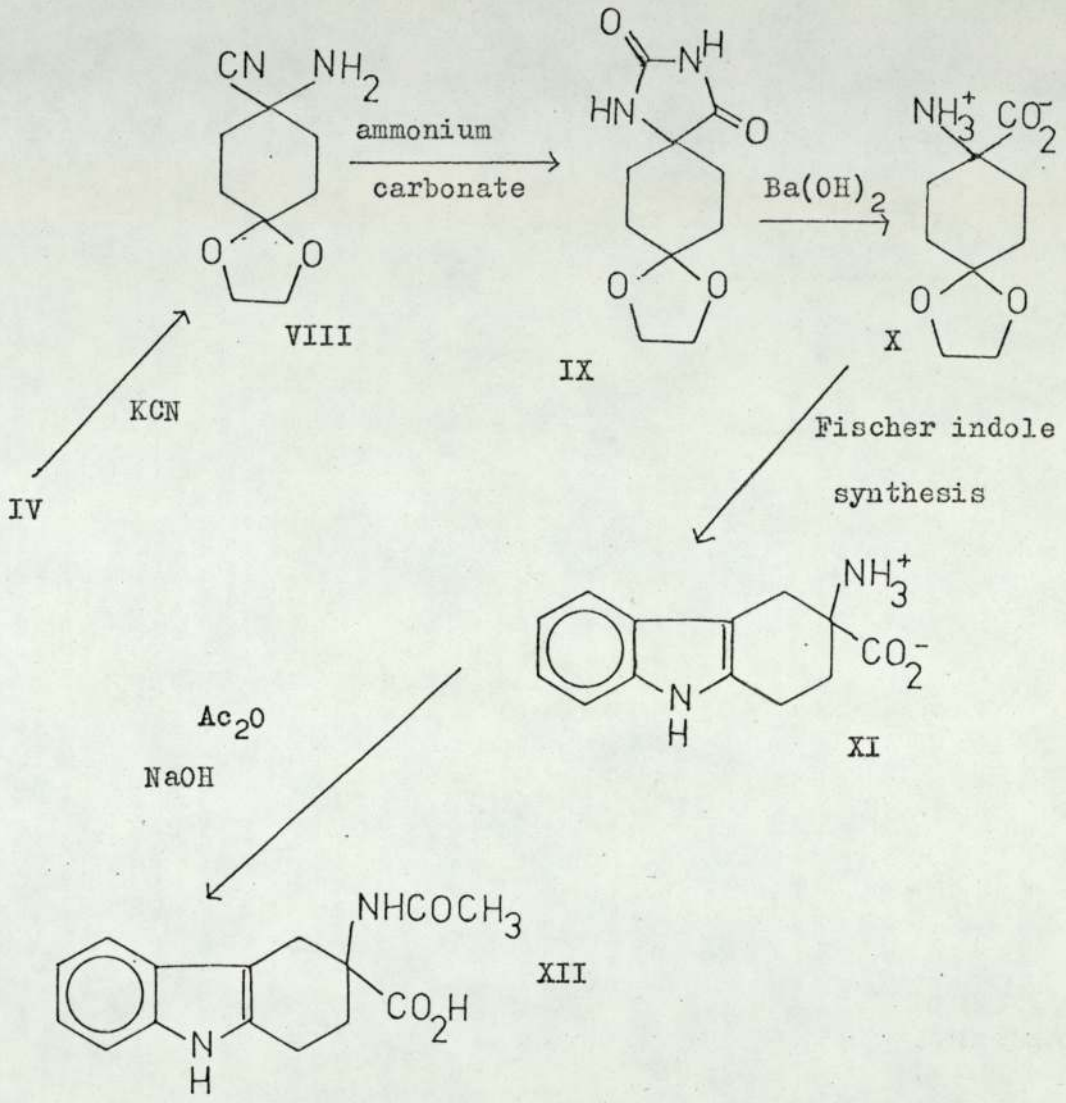
cyclohexanone (synthesised from quinitol according to Aldersley et. al.[57]) as follows:-



This route to the hydroxyketal VII gave higher yields than did direct formation from 4 - hydroxycyclohexanone.

A Strecker reaction on IV followed by hydrolysis via a hydantoin derivative (the Bucherer modification of the Strecker

synthesis[56]) led to the formation of the required amino acid X :-



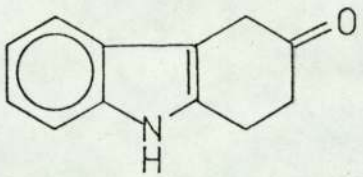
The direct hydrolysis of the aminonitrile proceeded only with difficulty, but with relative ease via the hydantoin IX which was hydrolysed by barium hydroxide. This procedure allowed the separation of inorganic reagent by precipitation with ammonium carbonate; taking the resulting solution to dryness gave amino acid X in high yield and purity. Other procedures gave lower yields because of losses due to the

high water solubility of X. Production of the hydantoin IX occurred in higher yields via the aminonitrile VIII than by direct formation from the ketone with ammonium carbonate and KCN; the Strecker reaction of the ketone IV with KCN, NH_4Cl and ammonia solution gave a good yield of aminonitrile VIII. Fischer indolisation of VIII gave the expected indolo - aminonitrile, but it was found difficult to hydrolyse this to the amino acid.

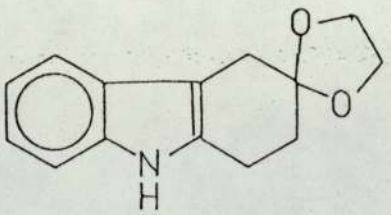
These compounds, and the ketones mentioned below, are also described elsewhere[58] .

(c) Ketones

(i) XIII



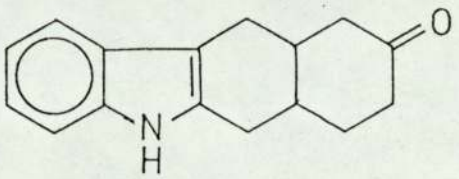
3 - hydroxy - 1,2,3,4 - tetrahydrocarbazole was synthesised by the method of Harley - Mason[59] , but attempts to oxidise this by various reagents including CrO_3/acetone, CrO_3/pyridine,[60] cerium salts,[61] two phase CrO_3,[63] sublimation with copper II oxide,[64] DMSO,[45,62] via the tosylate[46] or by the Oppenauer oxidation, failed. However, the Fischer indole synthesis on keto - ketal IV gave a 70% yield of ketal XIV when the catalysis conditions were such as to inhibit hydrolysis of the ketal, i.e. zinc chloride in dry



XIV

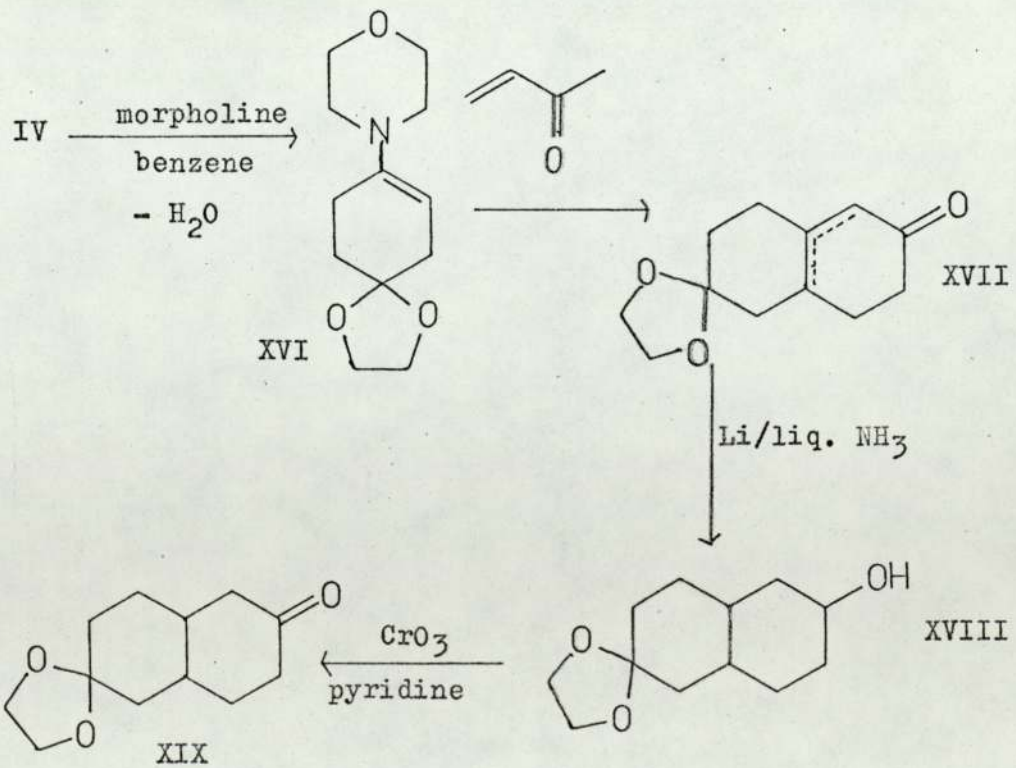
benzene. Aqueous solvents and acid catalysts gave yields less than 10%. Compound XIV was readily hydrolysed to XIII by aqueous HCl or a catalytic amount of p - toluenesulphonic acid in acetone.

(ii)



XV

The keto - ketal IV was further utilised to synthesise the monoethylene ketal of decalin - 2,6 - diome :-



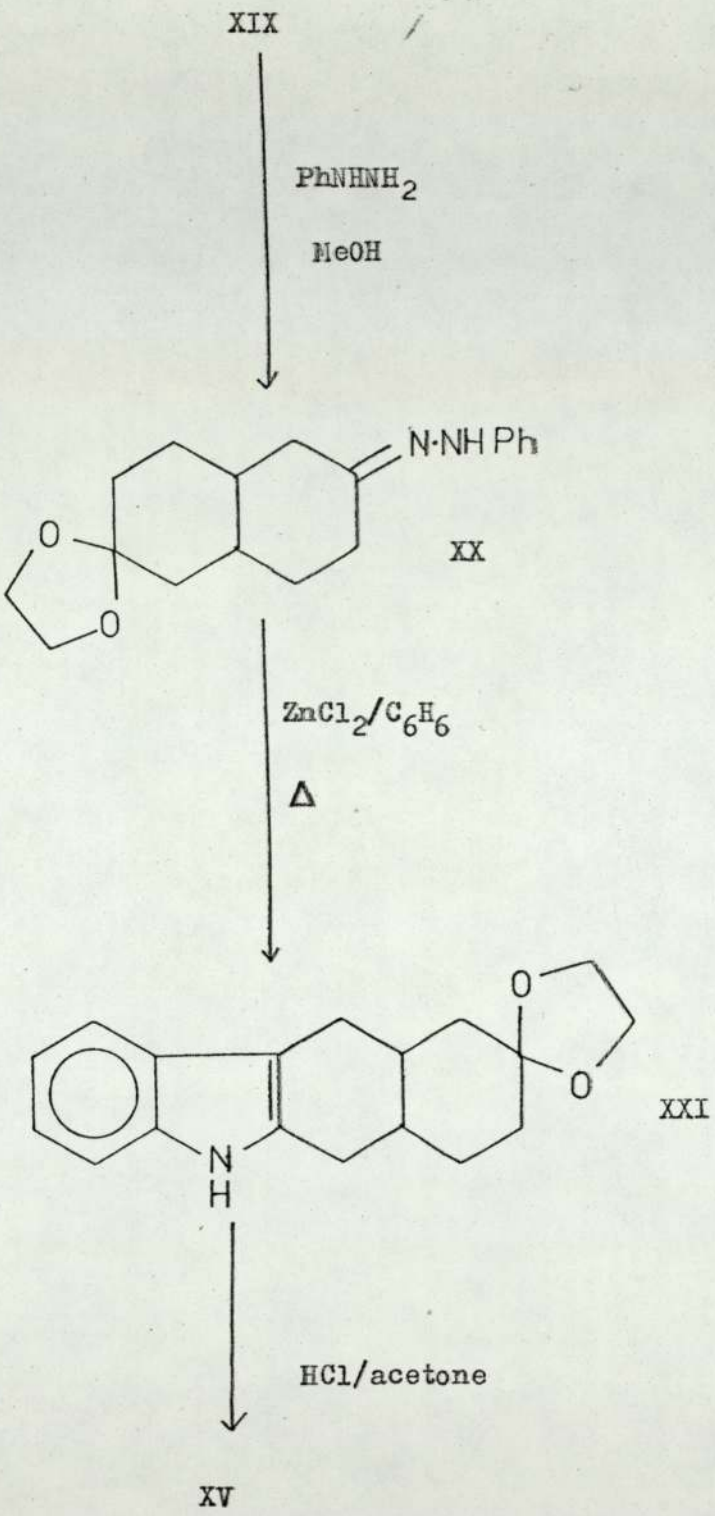
The enamine XVI was prepared following the method of Stork et. al.,[43] and was reacted immediately with methylvinylketone. The position of unsaturation of the ketone XVII produced was not determined, but it was reduced to alcohol XVIII. Under the conditions of reduction used here, several $\alpha\beta$- unsaturated ketones have been shown to give the saturated ketone.[65] However, infra-red analysis showed the product in this case to be hydroxylic, with no carbonyl group present. This may be an indication that compound XVII was $\beta\gamma$ rather than $\alpha\beta$ un - saturated with respect to the ketone.

Alcohol XVIII was oxidised in a similar manner to the reaction VII $\longrightarrow$ IV, using the CrO_3/pyridine complex in dichloromethane.[60] This yielded after workup a colourless oil with spectral characteristics expected of compound XIX.

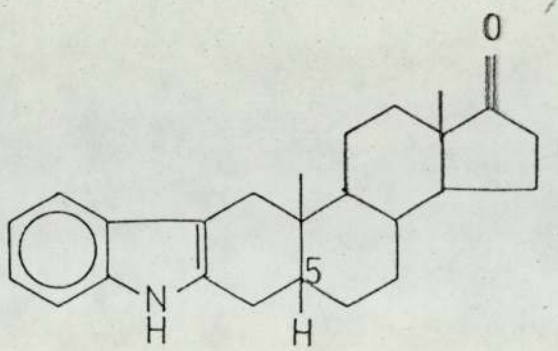
This oil was reacted with phenylhydrazine at room temperature; after workup, 14% yield of a white solid was obtained. No further crystallisation from the mother liquors could be induced, from which was inferred that the oil had reacted to give a mixture of isomeric phenylhydrazones, one of which had crystallised out.

This phenylhydrazone XX was indolised in anhydrous benzene with zinc chloride, giving a ketal XXI, which on hydrolysis gave the desired ketone XV.

The stereochemistry of fusion of the carbocyclic rings was not determined. The direction of indolisation was shown by mass spectrometry to yield the product shown. (sec. iv.)


XIX
PhNHNH2
MeOH
N·NH Ph
O
O
XX
ZnCl2/C6H6
Δ
O
O
N
H
XXI
HCl/acetone
XV

(iii)



XXII

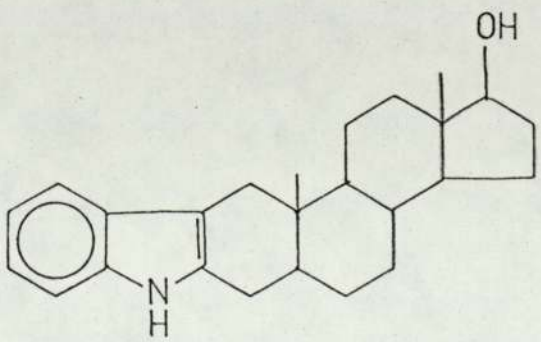
a 5α

b 5β

These indolosteroids were obtained in quantitative yield from a modified Fischer indole reaction on 5α and 5β - androstan -3,17 - diones. It was expected that because of the greater steric hindrance at the 17 position, the 3 - oxo group would be the more reactive. This was found to be so, for on dropwise addition of aqueous ethanolic phenylhydrazine hydrochloride to the steroid refluxing in ethanol, a precipitate of one pure product was produced within 5 minutes. The infra-red spectra showed the presence of an intact carbonyl group in each product, and the characteristic 3400 K indolic NH peak was also present.

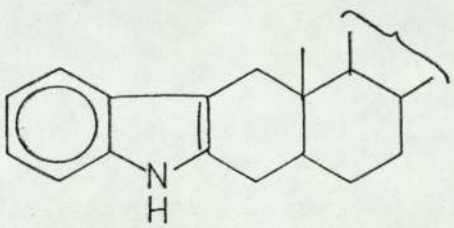
Again, mass spectrometry indicated the linear indole to be produced (vide infra). A related compound, the 17 - hydroxy indolosteroid XXIII was also made from 5α androstan -17 -β- ol - 3 - one by a similar technique.

XXIII

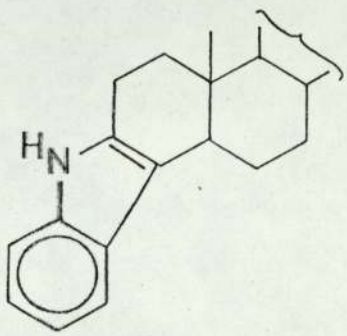


The Direction of Indolisation of the Polycyclic Ketones.

A number of indolosteroids have previously been prepared; indeed, the formation of a tetrahydrocarbazole derivative by reaction of a 3 - ketosteroid with phenylhydrazine was used as a means of characterisation of such steroidal ketones[88]. Doree and Petrow in 1935[88] concluded that their cholestanone tetrahydrocarbazole had the angular structure XXV, on the basis of surface area measurements and chemical analogy.



XXIV



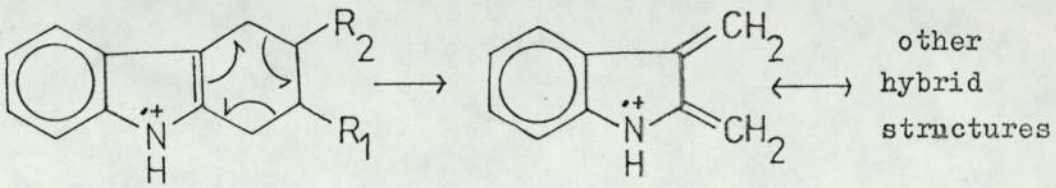
XXV

Since this work, several substituted indolosteroids were made by Lester et. al.[89]; they assumed the linear structures XXIV to be formed, without evidence or reference. Ban and Sato[91] showed by chemical degradation studies that 5α - cholestan - 3 - one gave the linear derivative, but the 5β ketosteroid gave instead the angular derivative XXV. This was reinvestigated by Harvey and Reid[90] who found that both derivatives were formed (as separated by GLC), in 90% : 10% ratios, the major component being that found by Ban and Sato.

Mass spectrometry was used here to determine the direction of indolisation. Structure XXIV is a 2,3 disubstituted

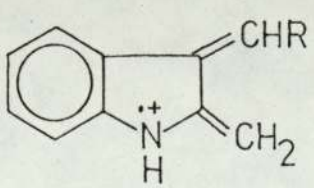
1,2,3,4 - tetrahydrocarbazole derivative, whereas structure XXV is 3,4 disubstituted. 2,3 disubstituted derivatives are expected[67] to fragment under electron impact by a reverse Diels - Alder pathway to give a base or intense peak at m/e 143:-

m/e 143



This fragmentation has previously been reported for tetrahydro - carbazole itself[66], and the cholestanone derivatives mentioned above[92], and a base peak of m/e 143 was observed for compounds XIV, XV, XXIIa and XXIIb. 3 - oxo - 1,2,3,4 - tetrahydro - carbazole had a base peak of m/e 156, with a relative abundance of 20% at m/e 143. Thus the expected loss[67] of ketene by the reverse Diels - Alder mechanism was apparently suppressed by the preferred formation of fragments of m/e 156 and 157(70%). Accurate mass determinations of these ions showed their compositions to be $C_{11}H_{10}N$ and $C_{11}H_{11}N$ respectively, corresponding to the loss of CO + H and CO respectively.

For the 3,4 disubstituted tetrahydrocarbazole derivative, the expected fragment would be:



m/e 142 + R

and an intense peak at m/e 143 would not be expected.

THE INDOLE CHROMOPHORE

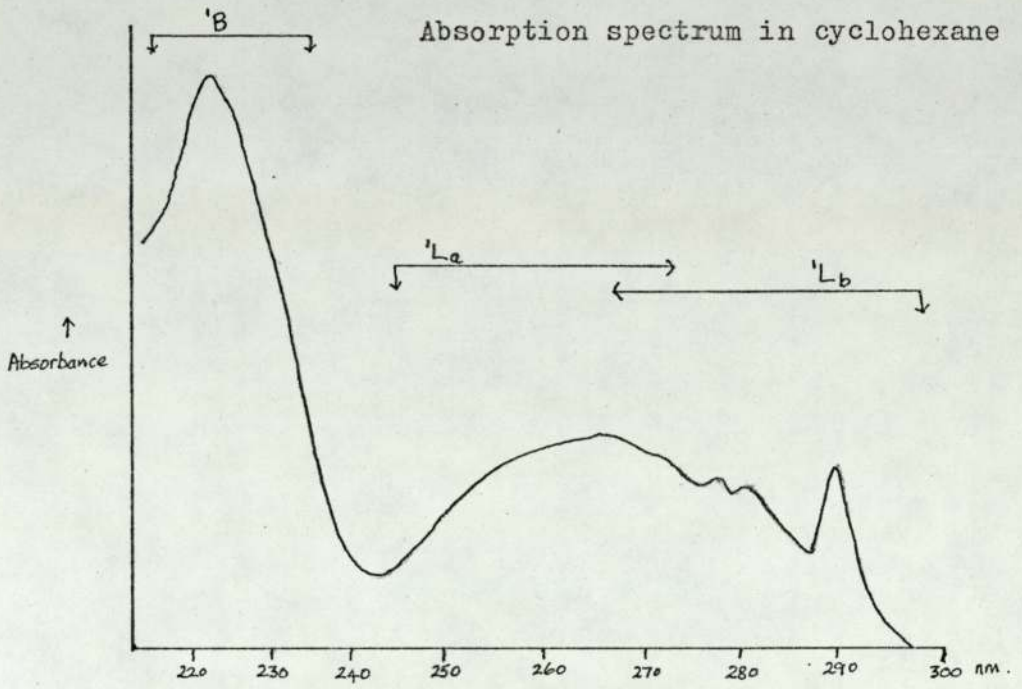
A. THE INDOLE CHROMOPHORE IN HYDROCARBON SOLVENTS

Introduction.

Fig. 1

The indole chromophore

Absorption spectrum in cyclohexane



That two electronic transitions are involved in the indole absorption band between 250 and 290nm (fig. 1) is a fact which has been appreciated for some time. However, as has been pointed out in chap. 1, there is controversy in the literature over the relative involvement of these two transitions in emission. Andrews and Forster in one of the most recent papers[30] point out that at ambient temperatures the ^1L_a and ^1L_b states will be in thermal equilibrium (in non polar solvents), this equilibration being faster than emission, and hence only one exponential decay is expected, this being from a (^1L_a + ^1L_b) equilibrated state mixture.

In order to interpret the fluorescence spectra and decay characteristics kinetically, it is necessary to analyse the total spectral curve into ^1L_a and ^1L_b components. For a single electronic transition, many compounds exhibit a mirror symmetry between absorption and emission curves[93]. (Deviation from this implies an alteration in equilibrium nuclear configuration on excitation). Thus, it should be possible to effect the separation of component bands either on the absorption or on the fluorescence spectrum. In the case of indole, molecular orbital calculations predict the bond lengths in the excited state to be different from those in the ground state[94], but no more so than in anthracene, a compound which shows mirror symmetry[93]. It therefore seems to be valid to assume that the ^1L_b contribution to the fluorescence spectrum of indole will be mirror symmetric with the ^1L_b contribution in absorption, and similarly for the ^1L_a contribution, although the total curves will not be mirror symmetrical because of pre-emissional thermalisation. Indeed, Walker et. al.[95] used the mirror symmetry relation to separate the bands in absorption by assuming emission to occur only from the ^1L_b state. These authors acknowledged that their values of the oscillator strengths of the two transitions were erroneous to some extent, as there was more overlap between the bands than they had assumed. This was earlier pointed out by Strickland et. al.[21] who had assigned peaks in the absorption spectra to ^1L_a or ^1L_b transitions on the basis of a solvent perturbation

technique. No estimates of the relative oscillator strengths were given, however.

Other attempts at analysis utilised polarisation data. Weber[19,20] examined viscous solutions and was able to show the presence of two transitions. No quantitative analysis was performed. Yamamoto and Tanaka[23] examined the polarised absorption spectra of indoles in the crystalline state, and gave values of oscillator strengths:

$f(^1L_b) = 0{\cdot}010, \qquad f(^1L_a) = 0{\cdot}112.$ (indole)

Interactions in the solid state are unlikely to be comparable to those in dilute solution, so the error involved in assuming these values to hold for dilute solutions is not known.

More recently, Strickland and Billups[22] found that the two transitions were already separated in 5 - methoxyindole, as judged by the solvent perturbation technique, i.e. the ^1L_b absorption band appeared at a longer wavelength than, and just removed from the ^1L_a band. They were able to measure the area under each band, giving oscillator strengths of:

$f(^1L_b) = 0.045, \quad f(^1L_a) = 0.138$ (5-methoxyindole)

Using their separated curves, they analysed the spectra of indole and 3 - methylindole, obtaining f - values of:

$f(^1L_b) = 0.019, \quad f(^1L_a) = 0.129$ (indole)

$f(^1L_b) = 0.027, \quad f(^1L_a) = 0.127$ (3-methylindole)

This work could be criticised on the grounds that the oxygen lone pair electrons in 5 - methoxyindole can interact rather strongly with the chromophore, and interaction may not be similar for both the ^1L_a and ^1L_b components. A methoxy

substituted indole is not a good model for an alkyl substituted indole such as tryptophan. Strickland and Billups pointed out that their oscillator strength values lie between those of Yamamoto and Tanaka given above, and those of Bernadin[96].

Molecular orbital calculations on indoles have been carried out by several workers. Some of the results obtained are tabulated below.

S_1 (^1L_b)		S_2 (^1L_a)			
Transition energy	f	Transition energy	f	method	ref.
4.52eV (274nm)	0.11	4.89eV (253nm)	0.18	PPP	106
36.7kK (272nm)	0.04	41.7kK (240nm)	0.21	PPP(LCAO)	24
4.45eV (279nm)	0.08	4.67eV (265nm)	0.12	limited CI	25
4.65eV (267nm)	0.06	4.73eV (262nm)	0.02	LCAO CI	107

Table 1 Calculated transition energies and oscillator strengths (f) for the first two excited singlet states of indole.

Whilst there is some order of magnitude agreement with the observed figures, the overall agreement is not good. Transition energies have been quoted in table 1 in nm as well as the original units, to aid comparison with fig. 1, and it would seem that little reliance can be placed on molecular orbital calculations for work of this type.

Before the paper of Strickland and Billups[22] appeared, work had begun here to attempt to separate the ^1L_a and ^1L_b transitions by a methyl substitution perturbation technique, which will now be described.

^1L_a - ^1L_b Analysis by Methyl Substitution Perturbation.

a) Theory.

Any perturbation technique for separating the components of a two component mixture relies on the differential effects of the perturbant on each component. Strickland's solvent perturbation technique[21], for instance, depends on the differential shifting of the ^1L_a and ^1L_b transitions by different solvents, methylcyclohexane and perfluorohexane. Whilst this gives good correlations for the peak positions, the resolution changes in different solvents, making difficult any assessment of the actual shape of the transitions, and hence their oscillator strengths. What is required is a method which differentially shifts the ^1L_a and ^1L_b absorptions without affecting the spectral resolution.

A superficial inspection of the absorption spectra of the methylindoles in cyclohexane reveals an essential similarity in overall shape, but some of the peaks are shifted differentially by positional substitution of the methyl group. Strickland's analysis of indole and 3 - methylindole implies that the vibrational peaks of the ^1L_b transition occur at the same relative positions in both compounds. Quantitative UV measurements show that the total oscillator strength of the 250 - 290nm band is similar in each methylindole[108]. So it seems reasonable to assume that changing the position of the methyl substituent mildly perturbs the indole chromophore by altering differentially the positions of the ^1L_a and ^1L_b absorptions. The actual shifts (ca. 2nm) are relatively very

small, but readily measurable.

Expressing these assumptions mathematically, the spectrum of a particular methylindole can be represented by a function F(x), where x is the abscissa variable, (in this case, wavelength) and F(x) is the relative absorption (or extinction coefficient). Then

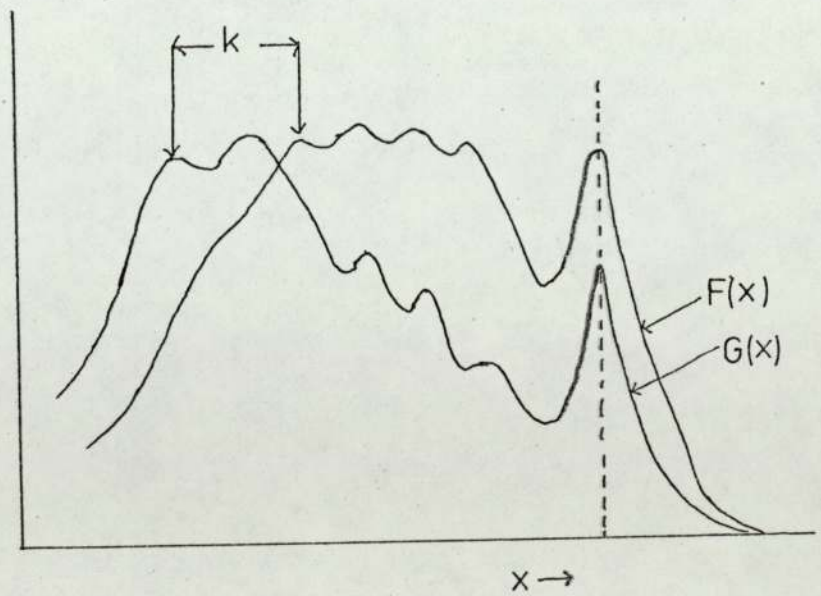
$$F(x) = A(x) + B(x) \qquad (1)$$

where A(x) and B(x) respectively represent the ^1L_a and ^1L_b components. If the wavelength origin is chosen arbitrarily, the ^1L_b transition of a second methylindole G(x) can be aligned with the ^1L_b transition in F(x), as Strickland has done in his diagrams[21]. Then the second methylindole is represented by:

$$G(x) = A(x - k) + B(x) \qquad (2)$$

where k is the separation of the ^1L_a transitions. (fig. 2)

Fig. 2



Subtracting eq. (2) from eq. (1),

$$H(x) = F(x) - G(x)$$
$$= A(x) - A(x - k) \qquad (3)$$

But

$$\left.\begin{array}{lll} H(x - k) = & A(x - k) - A(x - 2k) & \\ H(x - 2k) = & A(x - 2k) - A(x - 3k) & \\ \vdots & \vdots \quad \vdots \quad \text{etc} & \end{array}\right\} \qquad (4)$$

Noting that all these functions tend to zero as x approaches the limits of the extremities of the spectra, eqs. (3) and (4) may be added:

$$\sum_{i=0}^{n} H(x - ik) = A(x) \qquad (5)$$

where n is sufficiently large such that (x - nk) is outside the spectrum.

Thus A(x) is determined, and B(x) is obtained by simple subtraction:

$$B(x) = F(x) - A(x) \qquad (6)$$

In practical terms, this method was effected by digitising the spectra (solutions in cyclohexane, on a Beckman Acta V spectrophotometer) in 0.25nm steps downwards from 300 to 225nm. A program was written to align these spectra with respect to the ^1L_b 0 - 0 band, and then to carry out the above procedure, outputting the ^1L_a and ^1L_b components. A listing of the program appears in the appendix.

The digitisation of the 250 - 290nm band was complicated by overlap of the tail of the more intense ^1B absorption centred at ca. 220nm (see fig. 1). It was necessary to correct for

this, and this was done by curve fitting the ^1B band, and subtracting the fitted curve from the observed spectrum.

b) Curve Fitting of the Short Wavelength Absorption band.

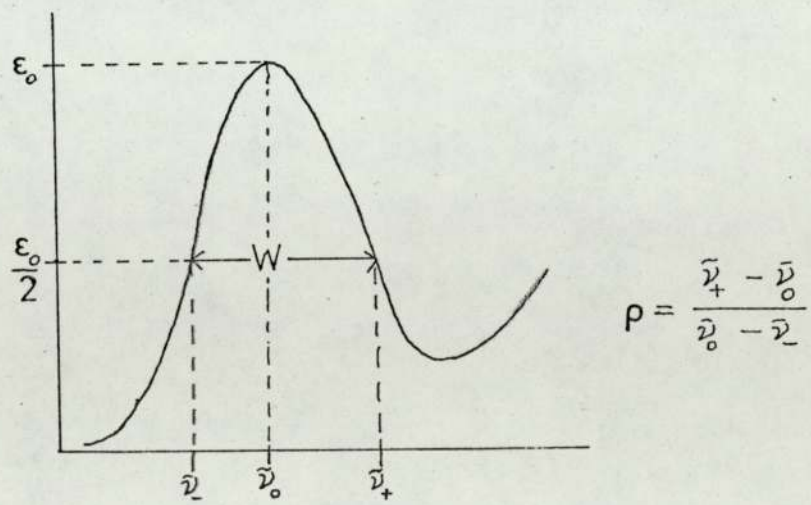
Absorption spectral curves have been fitted in the past to Gaussian or modified Gaussian curves[97,98], or convolutions of Gaussian and Lorentzian curves (the Voigt profile[99]). However, the type of curve at present giving the most successful fits to U.V. spectra appears to be the lognormal[102,105,110].

The lognormal distribution function has been defined by statisticians[100] as:

$$dF = \frac{1}{x\sigma\sqrt{2\pi}} \cdot \exp\left[-\frac{1}{2\sigma^2}(\ln x - \mu)^2\right] dx \qquad (7)$$

where σ and μ have the usual statistical meanings. Siano and Metzler reexpressed the lognormal equation in terms of more easily measurable parameters ε_o, $\tilde{\nu}_o$, W and ρ ; respectively the maximal extinction coefficient, the wavenumber of maximal absorption, the bandwidth at half maximal height and the skewness, defined as shown in fig. 3.

Fig. 3



Their resulting expression was:

$$\varepsilon(\tilde{\nu}) = \frac{\varepsilon_o b}{\tilde{\nu} - a} \cdot \exp(-c^2) \cdot \exp\left[-\frac{1}{2c^2}\left(\ln\frac{\tilde{\nu} - a}{b}\right)^2\right], \quad \tilde{\nu} > a$$

$$\varepsilon(\tilde{\nu}) = 0, \quad \tilde{\nu} \leqslant a$$

where $a = \tilde{\nu}_o - W\rho/(\rho^2 - 1)$

$b = W\rho/(\rho^2 - 1) \exp(c^2)$ (8)

$c = (\ln\rho)/(2 \ln 2)^{\frac{1}{2}}$

However, this was derived incorrectly, and Metzler later modified eqs. 8, obtaining[102]:

$$\varepsilon(\tilde{\nu}) = \frac{\varepsilon_o b}{\tilde{\nu} - a} \cdot \exp(-c^2/2) \cdot \exp\left[-\frac{1}{2c^2}\left(\ln\frac{\tilde{\nu} - a}{b}\right)^2\right], \quad \tilde{\nu} > a$$

$$\varepsilon(\tilde{\nu}) = 0, \quad \tilde{\nu} \leqslant a$$

where $a = \tilde{\nu}_o - W\rho/(\rho^2 - 1)$

$b = W\rho/(\rho^2 - 1) \cdot \exp(c^2/2)$ (9)

$c = (\ln\rho)/(2 \ln 2)^{\frac{1}{2}}$

I was unable to verify either of these expressions, but starting from the basic eq. 7, obtained eq. 10 (see appendix), which was used in this work. This was found to agree with a third version of Metzler's which he presented in a slightly simplified format[110].

as eq. (9), but where

$b = W\rho/(\rho^2 - 1) \cdot \exp(c^2)$ (10)

It was found to be impossible to fit this function to a spectral curve using a simple least - squares fitting procedure; the method was far too sensitive to small changes in any of the parameters. A damped least - squares method was used, as described by Pitha and Jones[103], who reviewed several methods

of optimisation of fit. The method adopted was their method V developed by Meiron[104], in which the least - squares iteration step is:

$$\underline{x}_{m+1} = \underline{x}_m - (\underline{B}_m + p.\underline{C}_m)^{-1}.\underline{G}_m \qquad (11)$$

where $\underline{x}$ is the vector of variables (parameters ε_o, $\tilde{\nu}_o$, W, ρ for each band);

$\underline{B}$ is the matrix given by

$\underline{B} = \underline{A}^T . \underline{A}$ where $\underline{A}$ is the matrix of partial derivatives $A_{jk} = \partial f_j / \partial x_k$, $(f_i = I_{obs_i} - I_{calc_i})$. and A^T is its transpose;

$\underline{G} = \underline{A}^T . \underline{f}$

$\underline{C}$ is the diagonal matrix formed from $\underline{B}$ with its off - diagonal elements zeroised;

p is the damping factor, set to give the lowest $\Sigma(f_i)^2$.

A program was written which would accept spectral data digitised in 1nm decrements from 300nm to 210nm or below, convert these to $(I_{obs}, \tilde{\nu})$ points, and then fit eq. (10), or a sum of bands each represented by eq. (10), to M of these points, where M could be any number of points (vide appendicem).

c) Results and Discussion.

Each of the methylindoles (1, 2, 3, 5 and 7) was fitted to a sum of 3 lognormals; 2 for the ^1B transition and 1 (intentially only a rough fit) for the ^1L_a - ^1L_b combination, as shown in fig. 4. Only the area of overlap, as shown, was fitted. The contribution of the ^1B peaks was then subtracted (manually) from the observed total spectrum, and the resulting ^1L_a + ^1L_b combination was used in the perturbation technique. Spectra

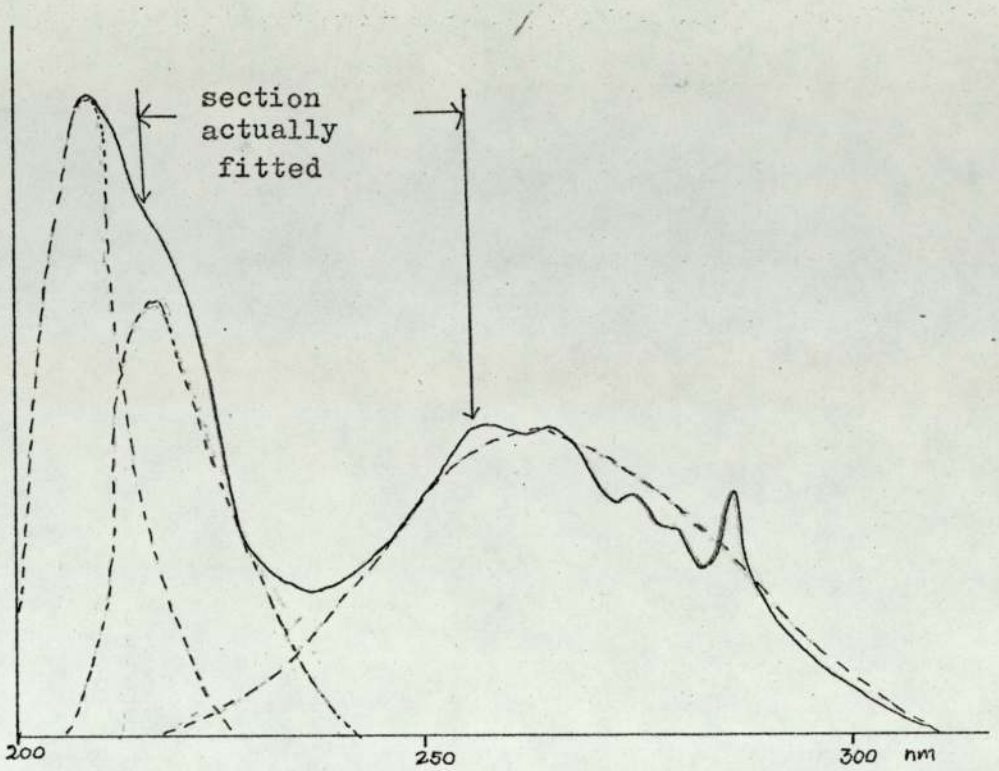


Fig. 4

Sketch showing the fitting of a sum of 3 lognormals.

were normalised in two ways, either by assuming the total oscillator strength to be identical for all the methylindoles, and normalising to equal areas under the curves, or by assuming the shortest wavelength peak or shoulder to be totally ^1L_a, and normalising to constant height of this peak. In calculation of the areas under a curve, a correction was performed for the error which would have been incurred because of linear wavelength rather than linear wavenumber plotting.

Consider now the original assumption of the technique, namely that the spectra of two methylindoles can be represented in the region under consideration by eqs. (1) and (2).

$$F(x) = A(x) + B(x) \qquad (1)$$

$$G(x) = A(x - h) + B(x) \qquad (2)$$

As has already been stated, the perturbation by a methyl group is small in terms of electronic spectral differences, but it is possible that the vibrational substructure could be altered sufficiently to cause large errors in the analysis. Examination of the I.R. spectra of the methylindoles reveals several similarities; all possess several strong, sharp bands between 700 and 800K; a broad medium band around 1000K, and fairly strong bands between 1400 and 1500K (KBr discs). If the vibrational properties of the ground electronic state have such similarities, then it is reasonable to expect the excited electronic states to be comparable also. In fact, Strickland[21] quoted ^1L_b bands at 0 - 0, 0 + 730, 0 + 980 and 0 - 760 K for both indole and 3 - methylindole. Thus it seems likely that

the bands should be the same shape for all the methylindoles. If there is a difference, then it can be represented mathematically as a third term in eq. (1), i.e.

$$F(x) = A(x) + B(x) + U(x) \qquad (12)$$

where U(x) is the difference term - the error in the original assumption. In the subsequent subtraction procedure, eq. (13) is obtained:

$$\sum_{i=0}^{n} H(x - ik) = A(x) + \sum_{i=0}^{n} U(x - ik) \qquad (13)$$

and if the error U(x) is a peak which is narrow compared to k, it is readily seen that the error will appear as a repetitive pattern in the plot of $\sum H(x - ik)$, with the repetition distance equal to k. Thus, if the error is peaky, it should be possible to see it, and visually subtract it out. One possibility is that the presence of a methyl group in a particular position may alter the allowedness of a particular vibronic transition, (e.g. the ^1L_a 0 - 0 transition), causing an error peak to appear in the subtracted spectra.

The combinations of methylindoles used here were 1/5, 2/5, 3/5 and 7/1, and the results obtained are presented in figs. 5 to 11.

Figs. 8 to 11 show that in fact there is considerable variation in the shapes of the ^1L_a and ^1L_b bands as the position of methyl substitution is varied. Nevertheless, the ^1L_b transitions possess similarities. A strong 0 - 0 band is indicated, with a less strong peak about 6nm to the blue (0 - 700K). Examination of the spectrum of 3 - methylindole

Fig. 5

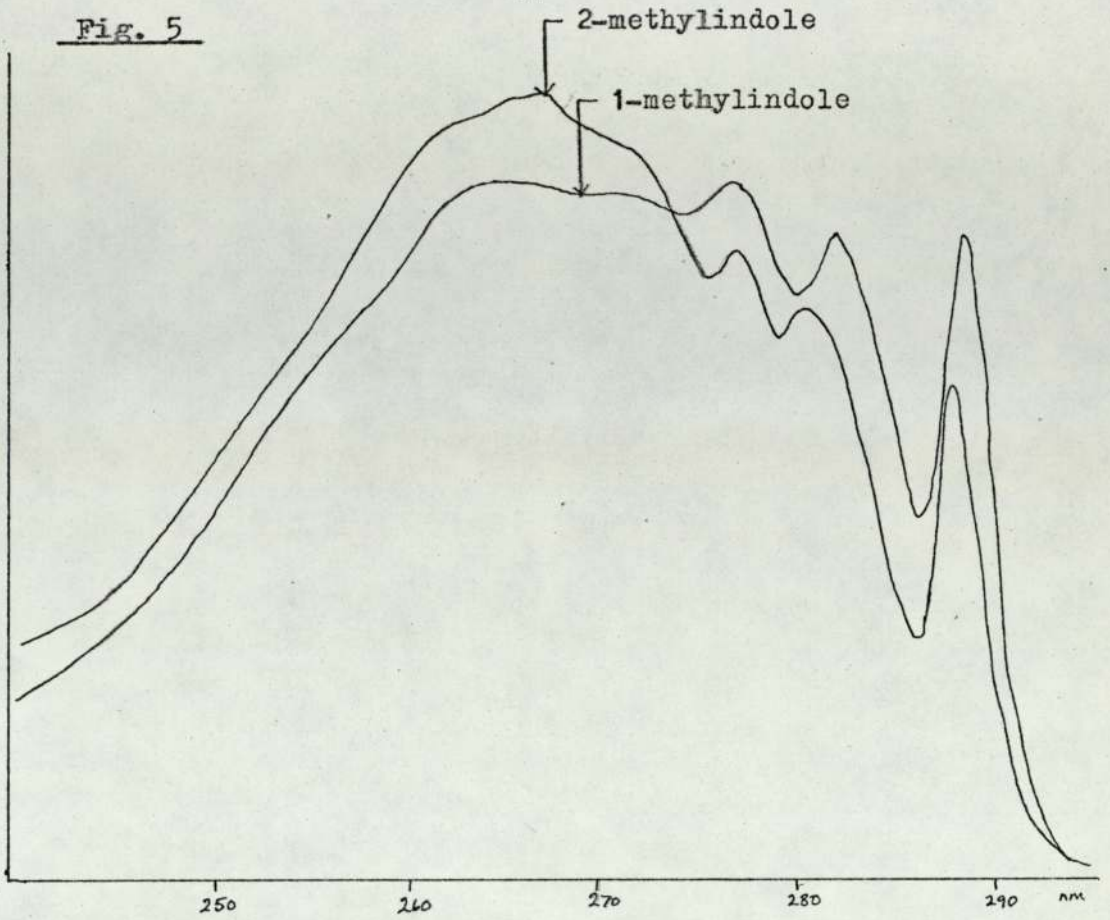

2-methylindole
1-methylindole
250
260
270
280
290
nm

Fig. 6

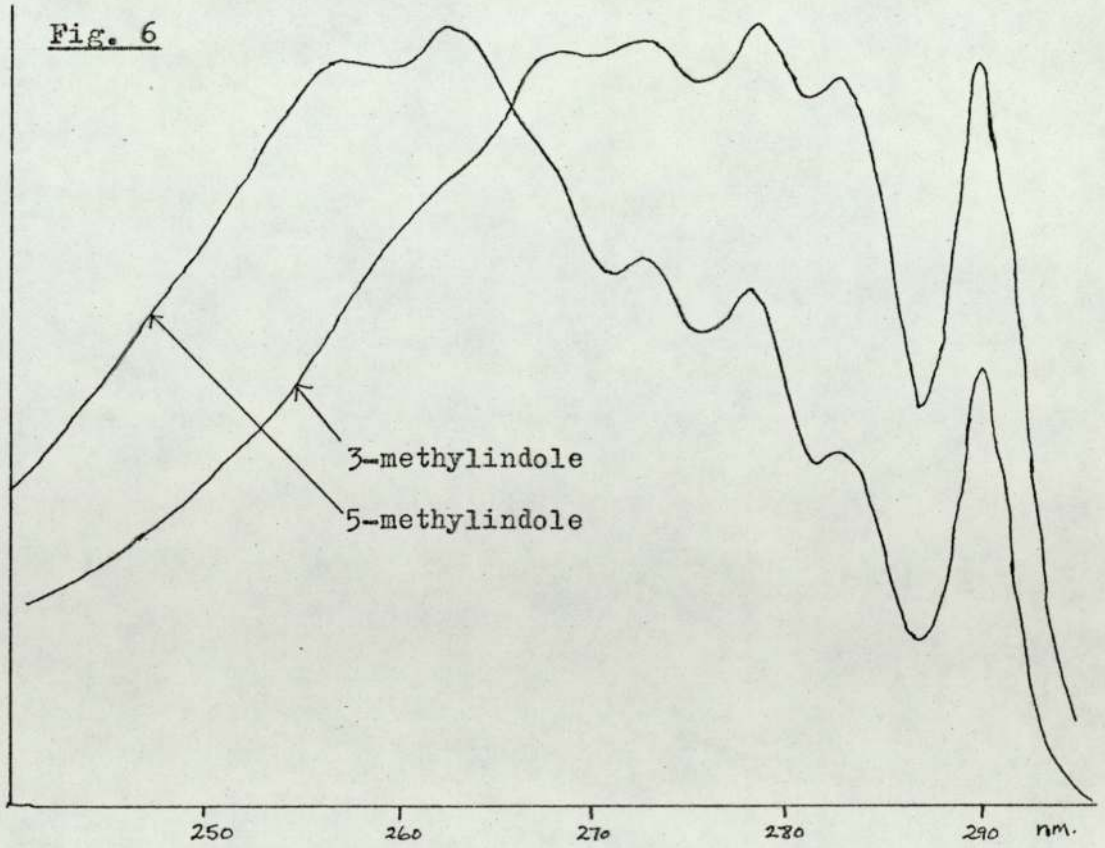

3-methylindole
5-methylindole
250
260
270
280
290
nm.

Fig 7.

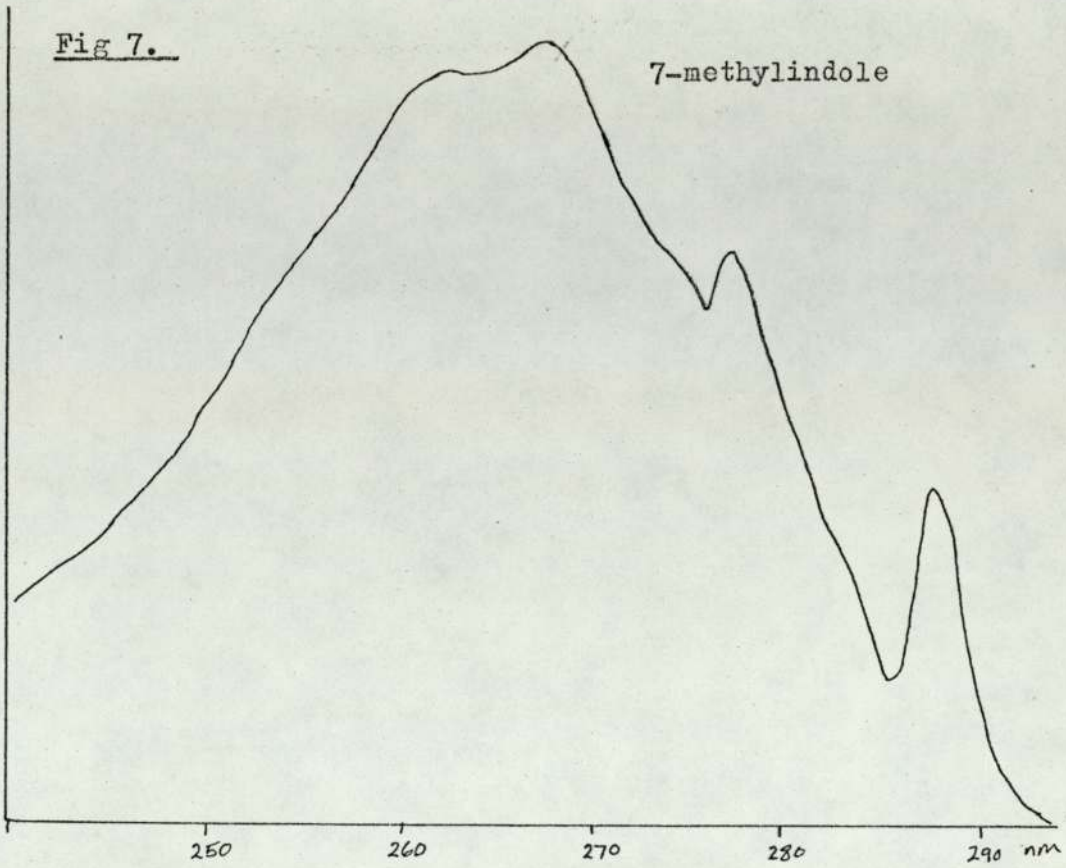


Fig. 8.

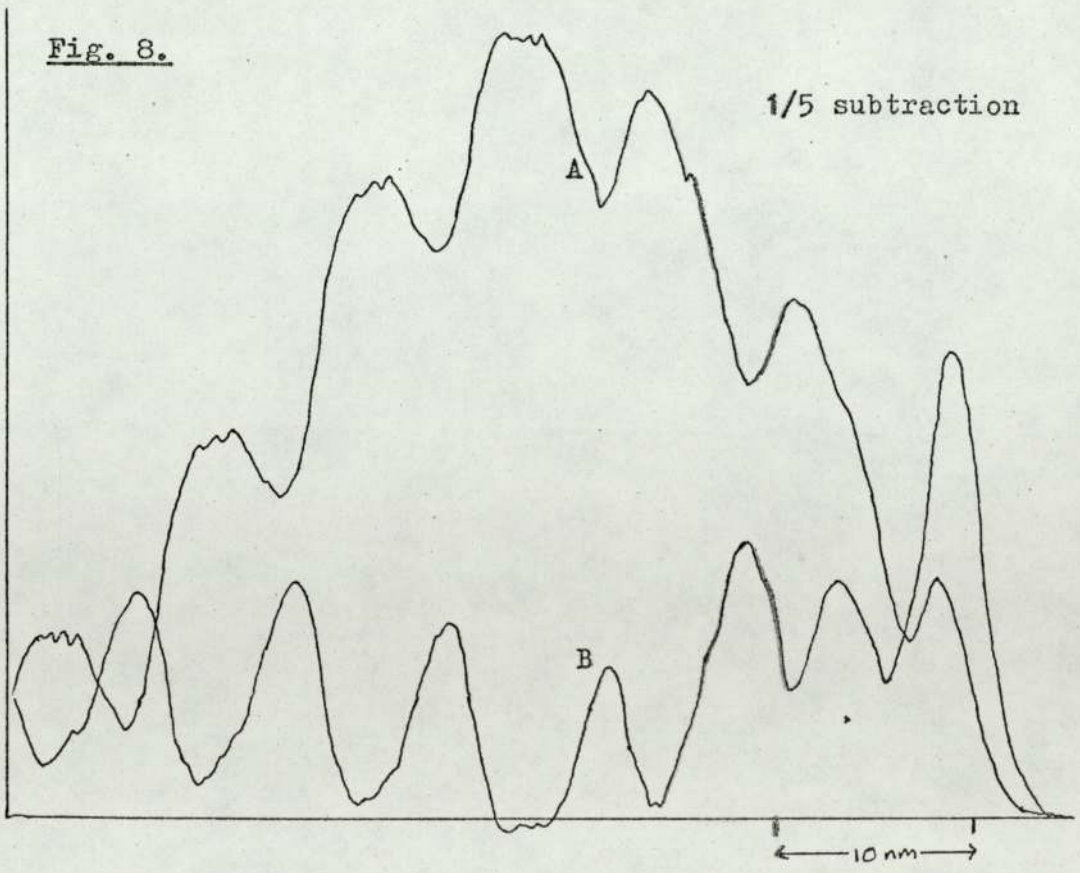


Fig. 9

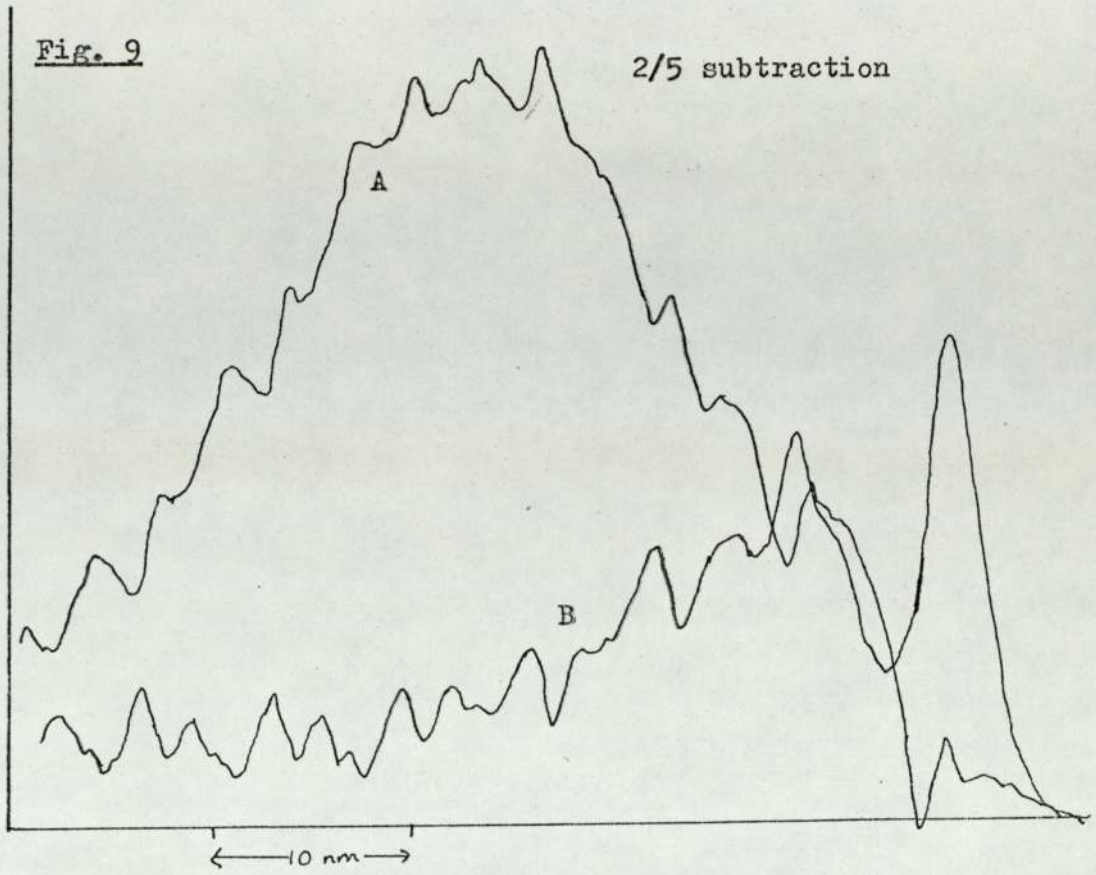


Fig. 10

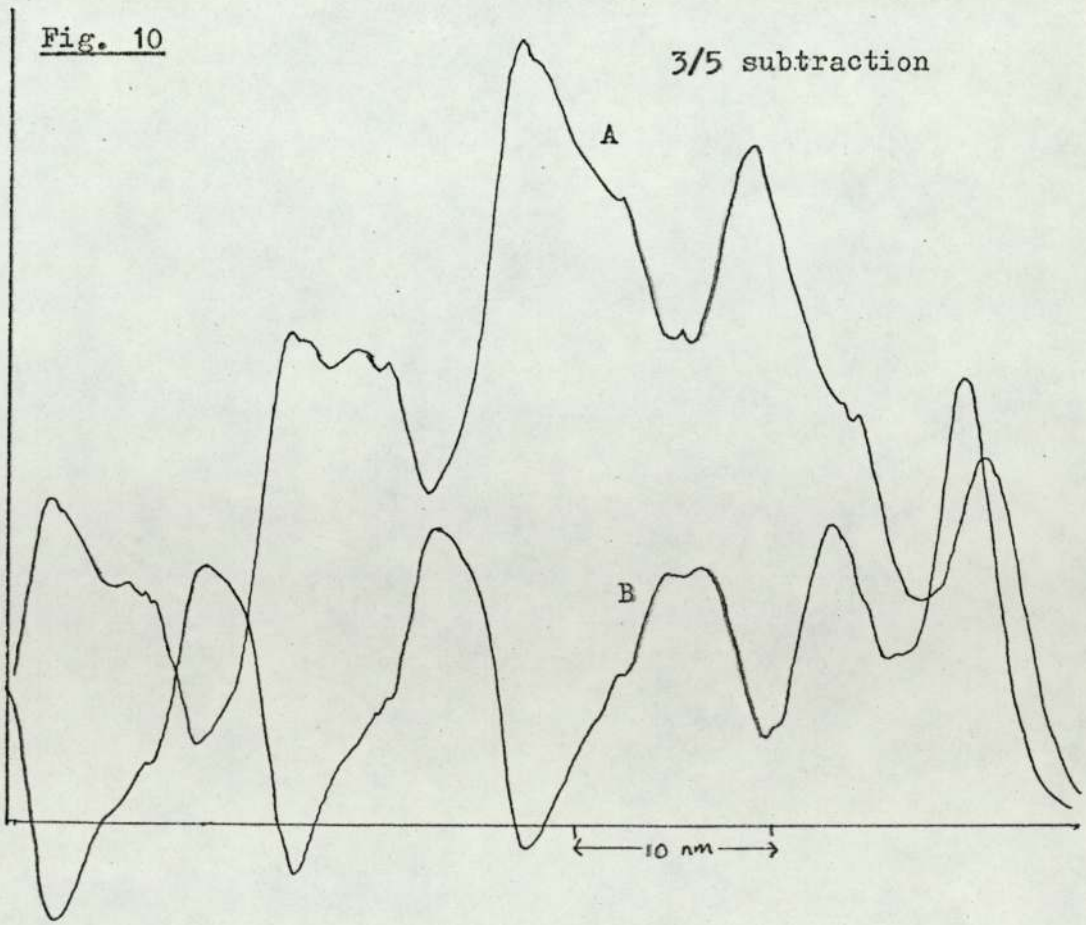


Fig. 11 7/1 subtraction

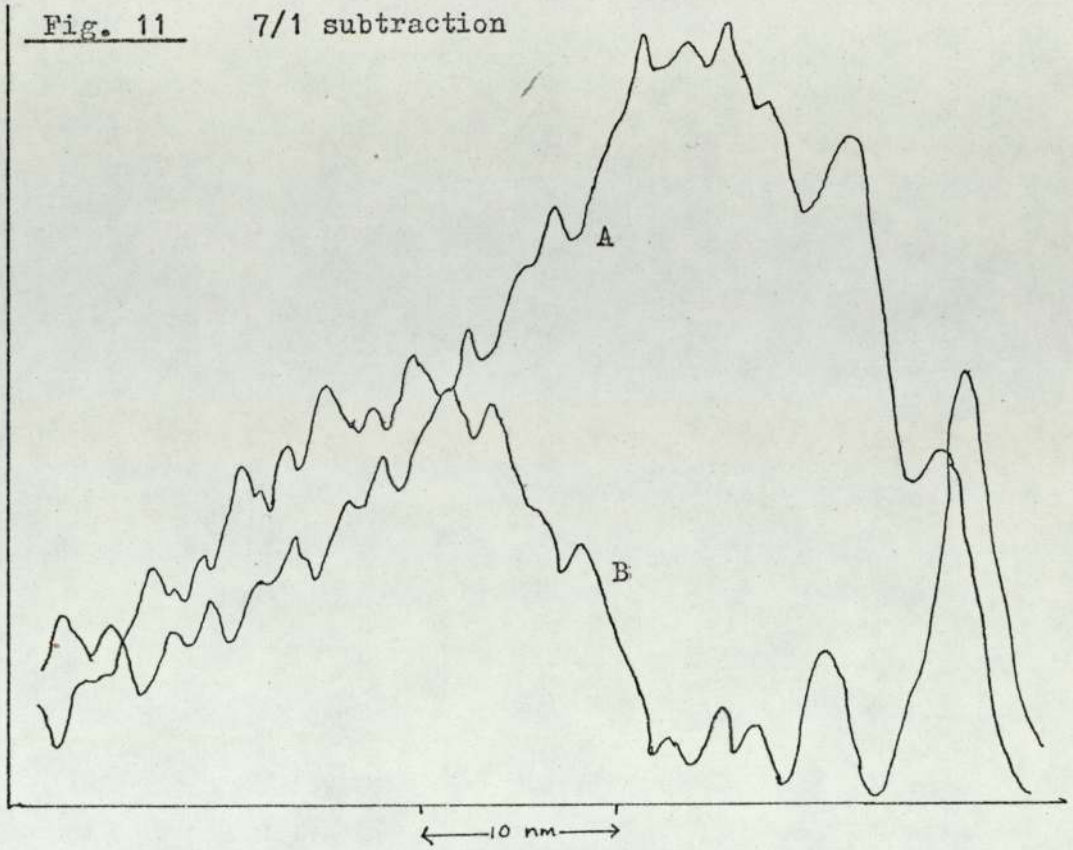
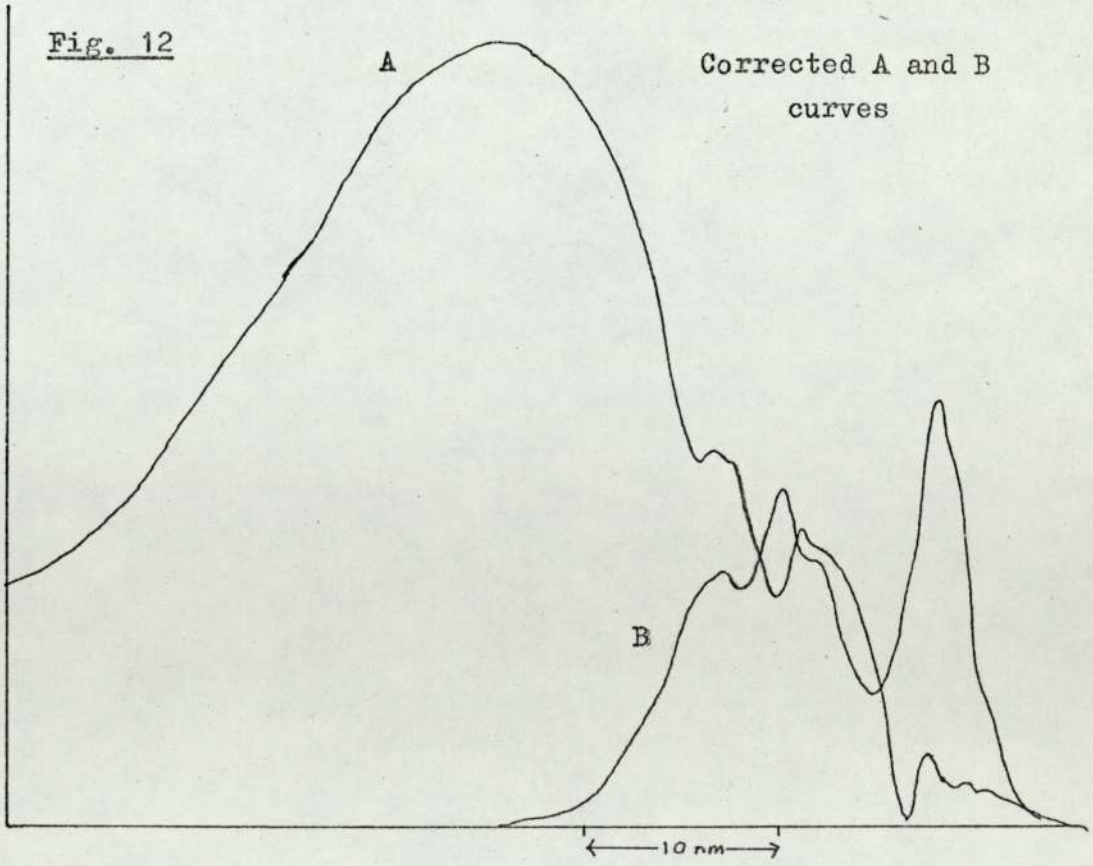


Fig. 12



Corrected A and B curves

(fig. 6) reveals a particularly strong band at the 0 - 0 ^1L_b position, which Strickland[21] assigned to an overlap of 0 - 0 ^1L_a and 0 - 0 ^1L_b. The ^1L_a transitions calculated here show only a weak 0 - 0 band except in the 3/5 comparison case. It seems likely, therefore, that a methyl group in the 3 position increases considerably the intensity of the ^1L_a 0 - 0 band, and that this causes the large periodic error structure in the 3/5 comparison (Fig. 10).

Again, 1 - methylindole (fig. 5) has an intense 0 - 0 band, which manifests as a periodic error in the 1/5 comparison. (fig. 8).

2 - and 5 - methylindoles seem to be more compatible in band shape. Fig. 9 has the general shape expected from previous work for the two transitions. Again, a periodic error is superimposed, but it is more easily corrected for. The 2/5 comparison case was, in fact, used to calculate the relative oscillator strengths. The transitions as presented in fig. 9 were visually corrected for the periodic error; the areas were calculated using conversion to a wavenumber scale, and the relative oscillator strengths were thus obtained. In the 2/5 comparison, the sum of $^1L_a + {}^1L_b$ is equivalent to the spectrum of 2 - methylindole. The total oscillator strength of 2 - methylindole was obtained by integrating the $^1L_a + {}^1L_b$ spectrum with respect to wavenumber, normalising the 288nm peak to an extinction given by $\log \varepsilon = 3.67$ (108), then using the relation

$$f = 4.319 \times 10^{-9} \int_{\tilde{\nu}} \varepsilon \, d\tilde{\nu} \quad \text{(ref. 109)} \qquad (14)$$

The results obtained are given below.

Total oscillator strength ($^1L_a + {}^1L_b$) for 2 - methylindole

$= 0.168$

$f(^1L_a) = 0.123$

$f(^1L_b) = 0.045$

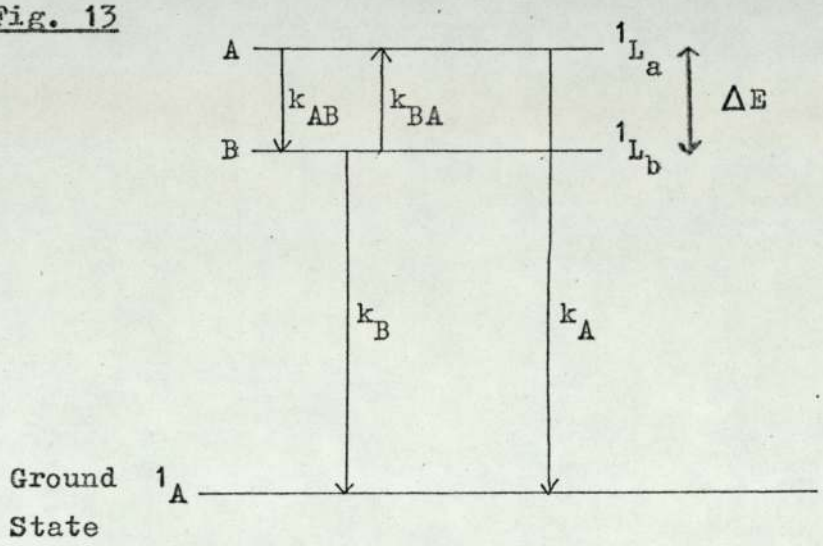
Fig. 12 shows the corrected ^1L_a and ^1L_b transitions from the 2/5 comparison.

These results agree with those of previous workers to an order of magnitude. The method shows that the shapes of the ^1L_a and ^1L_b transitions change on methyl substitution, hence the figures obtained only approximate to the values for any single indole, and represent an average for the indole chromophore. Because of the magnitude of the periodic error, it is not considered that the results are likely to be any more accurate than those of previous workers.

The Indole Chromophore : Fluorescence.

From a consideration of the absorption spectra, a simple energy level diagram may be constructed for the indole chromophore (fig. 13)

Fig. 13



where k_A, k_B are fluorescence rate constants; k_{AB}, k_{BA} are thermalisation rate constants; non - radiative processes omitted. A and B denote ^1L_a and ^1L_b respectively, and square brackets denote level populations or concentrations.

The order and separation of the A and B levels will vary depending on the substitution pattern. The thermalisation constants k_{AB}, k_{BA} are related according to the Boltzmann distribution:-

$$\frac{[A]}{[B]} = K = \frac{k_{BA}}{k_{AB}} = \exp^{-\frac{\Delta E}{kT}} \qquad (15)$$

and k_{AB}, $k_{BA} \gg k_A$, k_B .

Consideration of the kinetics of this system yields the equation given by Andrews and Forster[30]

$$k_{obs} = x_A k_A + x_B k_B \qquad (16)$$

where x_A, x_B are mole fractions of A and B, and k_{obs} is the observed decay constant. Assuming that the ratios of transition probabilities for the $^1A - {}^1L_a$ and $^1A - {}^1L_b$ are the same for both emission and absorption[30,93], then

$$\frac{k_A}{k_B} = \frac{f(^1L_a)}{f(^1L_b)} \qquad (17)$$

which, using the figures obtained above, gives $k_A/k_B \doteq 3$.

Using Strickland's analysis[21], in indole, A is 974K above B. (= 11.66 $kJ.mol^{-1}$). At 300°K, kT = 2.49 $kJ.mol^{-1}$. Then

$$\frac{[A]}{[B]} = \exp\left(-\frac{11.66}{2.49}\right) \doteq 0.01$$

i.e. 1% of the excited state population immediately prior to emission is [A], 99% [B].

Now $$k_{obs} = \frac{Q}{\tau_F} \qquad (18)$$

where Q is the quantum yield of fluorescence. For indole, $Q = 0.59$[30] and $\tau_F = 9 \times 10^{-9}$ s [30].

$$\therefore \quad k_{obs} = 6.5 \times 10^7 \ s^{-1}.$$

Substituting in eq. (16), with $k_A = 3k_B$,

$$6.5 \times 10^7 = (0.01 \times 3k_B) + 0.99k_B$$

$$\underline{k_B = 6.4 \times 10^7 \ s^{-1}}$$

and $$\underline{k_A = 19.2 \times 10^7 \ s^{-1}}$$

The validity of these constants may be checked by using them to calculate the lifetimes of other indoles. From the coincidence of the 0 - 0 bands in skatole, $x_A = x_B$ for skatole; 2,3 - dimethylindole has A 450K below B[30,21]. These figures lead to the predicted lifetimes shown in table 2.

Table 2

Compound	A − B (K)	$\therefore k_{obs}$ (s^{-1})	Q	$\therefore \tau_F$ (ns) calc.	τ_F (ns) obs.
Indole (reference)	+974	(6.5×10^7)	0.59		9
2,3 - dimethylindole	-450	18.1×10^7	0.40[30]	2.2	2.2[30]
3 - methylindole	0 ± 120	$12.8 \times 10^7 \pm 1.8$	0.42	3.3 ±0.5	4.6[30]

The reciprocals of the observed lifetimes give the total decay constants, i.e.

$$1/\tau_{F_{obs}} = k_{obs} + k_{NR_{obs}}$$

$$= x_A(k_A + k_{NR_A}) + x_B(k_B + k_{NR_B})$$

For a molecule in a solvent which is only weakly interacting, the rate of internal conversion is expected to be low[93,p.146], so that k_{NR} is a good approximation to the intersystem crossing rate constant k_{ISC}. Thus, the values for k_{ISC} may be calculated; some values obtained are quoted in table 3. It was attempted to obtain some of these figures experimentally, without success (see the discussion on carbonyl effects, and the experimental section).

Table 3 Intersystem crossing rates

Compound	$1/\tau_{F_{obs}}$	k_{obs}	$\therefore k_{ISC_{obs}}$
Indole	11.1×10^7	6.5×10^7	4.6×10^7
3 - methylindole	21.8×10^7	12.8×10^7	9.0×10^7
2,3 - dimethylindole	45.5×10^7	18.1×10^7	27.4×10^7

The lower excited singlet states of indole in a weakly interacting solvent such as cyclohexane may be illustrated summarily as in fig. 14.

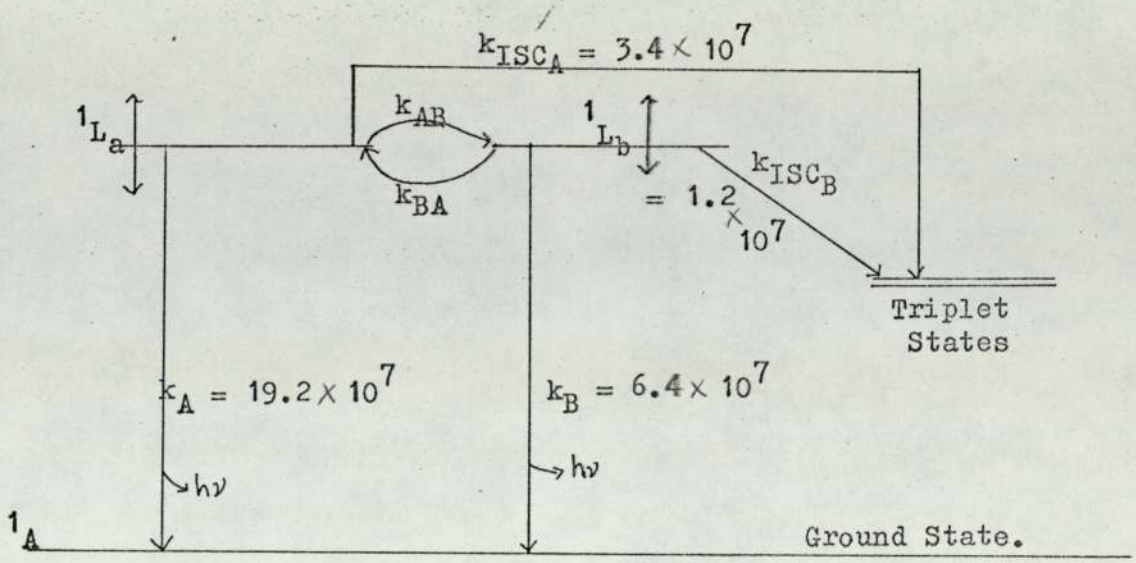

$k_{ISC_A} = 3.4 \times 10^7$
^1L_a
k_{AB}
k_{BA}
^1L_b
k_{ISC_B}
$= 1.2 \times 10^7$
Triplet States
$k_A = 19.2 \times 10^7$
$k_B = 6.4 \times 10^7$
$h\nu$
$h\nu$
^1A
Ground State.

Fig. 14

B. SOLVENT EFFECTS ON THE INDOLE CHROMOPHORE.

The effect of different solvents on the fluorescence and absorption band maxima of indole has been known for some time[37]. There is only a small effect on the absorption maximal wavelength, whilst the emission spectrum in more polar solvents is red - shifted. However, there is controversy in the literature as to the explanation of this phenomenon.

One of the most comprehensive theoretical studies of solvent effects on electronic spectra was that of McRae[111], who derived a general expression for the solvent induced frequency shift by means of second order perturbation theory. He considered a solute molecule to be reducible to a point dipole in an environment of an isotropic dielectric solvent. He utilised also Onsager's reaction field theory, which gives the reaction field due to the solvent dielectric as

$$R = \frac{2(M + \alpha R)}{a^3}\left[\frac{2(D - 1)}{2D + 1}\right] \qquad (19)$$

where M is the solute dipole moment; D is the solvent static dielectric constant; α is the solute polarisability. Then assuming that $\alpha \simeq a^3/2$ (ref.111), and including the inductive polarisation where D is replaced by n_o^2 (n_o is the solvent refractive index extrapolated to zero frequency),

$$R = \frac{2M}{a^3} \cdot \left[\frac{D - 1}{D + 2} - \frac{n_o^2 - 1}{n_o^2 + 2}\right] \qquad (20)$$

Using this expression, perturbation theory yielded the frequency shift:-

$$= (A+B+C)\frac{(n^2-1)}{(2n^2+1)} + E_F\left\{\frac{D-1}{D+2}\frac{n^2-1}{n^2+2}\right\} + F_F\left\{\frac{D-1}{D+2}\frac{n^2-1}{n^2+2}\right\}^2 \qquad (21)$$

where A, B, C, E, F are constants for a particular solute, and n is the refractive index at the sodium D line frequency. Subscript F refers to fluorescence; a similar expression was given for absorption, with E_F, F_F replaced by E_A, F_A. This derivation has also been discussed in detail by Mataga and Kubota[112].

Prior to this work of McRae, Kirkwood had given a formula for dipolar interactions, which he assumed to be represented by a quantity K, where

$$K = \frac{D - 1}{2D + 1} \qquad (22)$$

This has been discussed with other expressions, by Katritzky et. al.[113]. If the refractive index term is included in eq. (22), the orientation polarisation formula used by Mataga et. al.[31], and Lippert[114], is obtained:

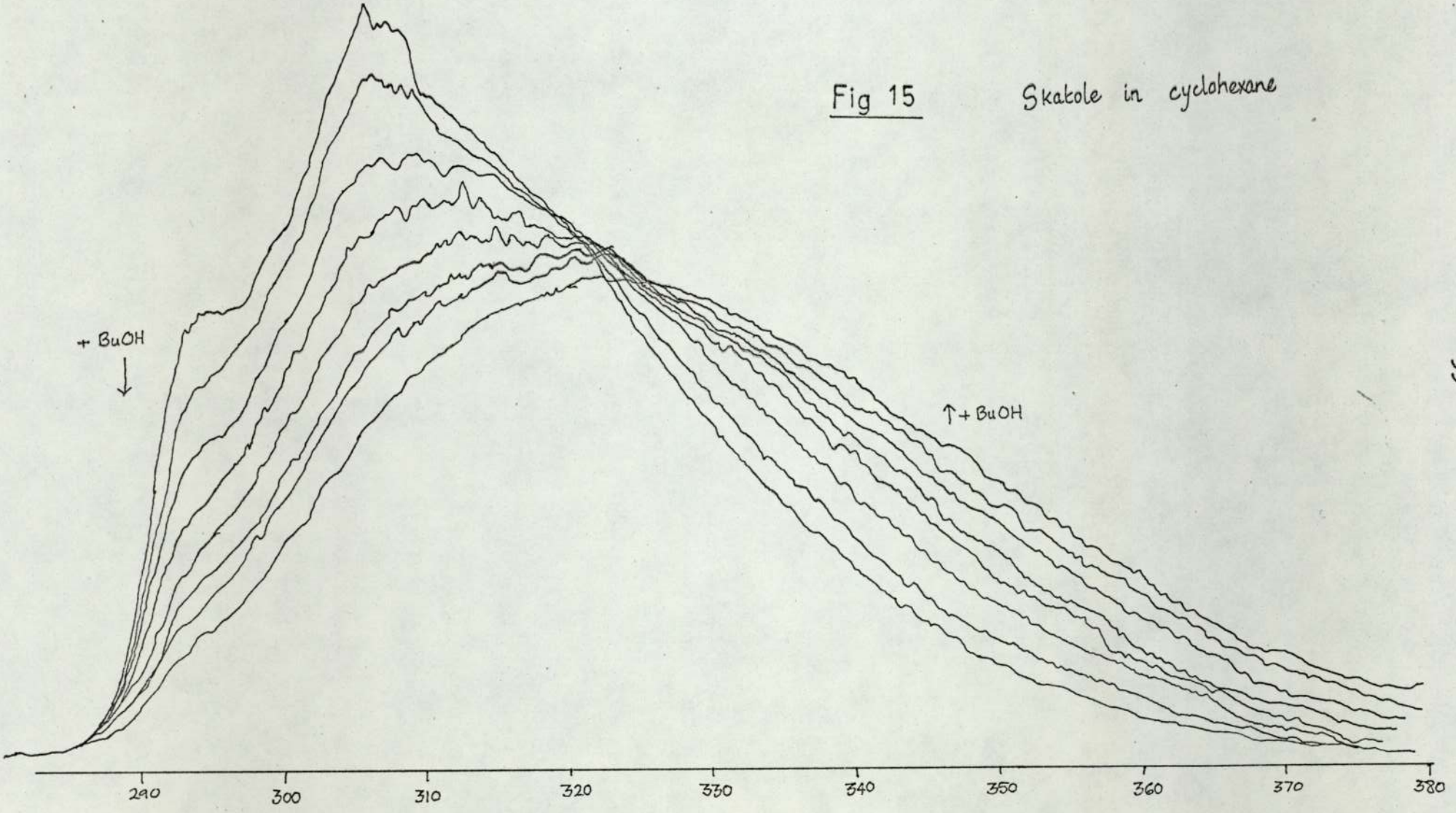
$$K' = \frac{D - 1}{2D + 1} - \frac{n^2 - 1}{2n^2 + 1} \qquad (23)$$

and the frequency shift is given by

$$\nu_A - \nu_F = \frac{2(M_{exc} - M_{gnd})^2}{R^3 hc} \cdot K' + \text{const.} \qquad (24)$$

This equation was tested for the case of indole in a number of solvents by Mataga et. al.[31], and also by Walker et. al.[32]. A poor correlation was found for polar solvents, where the red shift was anomalously large. This was interpreted by Mataga as being the result of a large increase in dipole moment on excitation, giving a greater solute - solvent stabilisation in the fluorescent state than in the ground and Franck - Condon excited states. Furthermore, it was suggested that the ^1L_a state was considerably more stabilised by

Fig 15 Skatole in cyclohexane



solvent interaction than the ^1L_b state, resulting in a reversal of levels, this explaining the change in band shape which accompanies the red shift.

Against this theory, Walker et. al.[32] found that addition of only a small amount of ethanol or butanol to a cyclohexane solution of indole caused a large red shift, where the change in bulk dielectric properties was insignificant. They postulated the formation of complexes between solvent and solute, i.e. the emitting species was thought to be not a single indole molecule, but a stoicheiometric complex between indole and solvent. Eisinger and Navon[33], on the other hand, preferred the view that in non polar solvents, a small amount of polar solvent would aggregate around the indole, i.e. the micro dielectric environment of the indole would be different from the bulk dielectric state.

Walker's kinetic analysis[32] gave 2 alcohol molecules per indole (using indole, 1 - methylindole, or 1,3 - dimethylindole with butanol or methanol) in the exciplex. Later investigations by Longworth[115] indicated that the indole - isopropanol exciplex had 1 : 1 stoicheiometry. Titration of 3 - methylindole in cyclohexane with 1 - butanol solution was performed in this laboratory, and a typical set of spectra obtained during titration is shown in fig. 15.

The apparent isoemissive point indicates that two species only are present, i.e. if a 1:2 exciplex is formed, then there is no detectable 1 : 1 intermediate. In order to determine the stoicheiometry, values of I_E/I_M (where I_E is

the exciplex emission intensity and I_M is the unexciplexed (monomer) emission intensity) must be plotted against the alcohol concentration, as shown by Walker et. al.[32]. The method of obtaining I_E/I_M requires some comment.

Walker's formula:

$$\frac{I_E}{I_M} = c \cdot \frac{i_2}{i_1}$$

(where c is a constant and i_1, i_2 are signal readings at wavelengths 1 and 2) - requires the choice of two reference wavelengths, one at which the exciplex intensity is ca. 0, and the other at which the monomer has approximately zero intensity. This was derived by Stevens and Ban[122]. But these spectra overlap to such an extent that this requirement cannot be met, and hence Walker's values for I_E/I_M are inaccurate to the extent produced by this error.

Whatever the method used to obtain I_E/I_M, the unperturbed spectra of monomer and exciplex must be known. The monomer spectrum can be obtained easily - it is the spectrum in the absence of any alcohol. The exciplex spectrum is not so easily obtained. Walker quotes two methods -

i) sufficient alcohol is added to convert much of the indole to exciplex, but insufficient to cause a dielectric shift, or

ii) A much higher alcohol concentration is used, and a correction is applied for the dielectric induced red shift.

The agreement of these two methods was quoted at $\pm$2nm in the peak maximum of the exciplex. However, examination of the exciplex spectrum in fig. 15 shows that if the curve is

shifted by 2nm in either direction, the emission intensity at 295nm (equivalent to the 290nm wavelength used for indole by Walker) changes by a considerable percentage of its absolute value. This error occurs regardless of the method of calculation used.

The exciplex spectrum used here was taken to be the last spectrum produced during the titration after which further addition of alcohol did not alter its shape, but merely shifted it to the red.

Then, considering wavelengths of 295 and 340nm,

for the skatole exciplex, $\frac{i_{295}}{i_{340}} = 0.22 \pm 0.15$

for skatole monomer, $\frac{i_{295}}{i_{340}} = 2.32$

Mixtures of exciplex and monomer produced during the titration will have (after correction for dilution, which was only 4% at the end of the addition of alcohol) values of $\frac{i_{295}}{i_{340}} = x$ between these values, and it can easily be shown that

$$\frac{[\text{exciplex}]}{[\text{monomer}]} = \frac{2.32 - x}{x - 0.22} \propto \frac{I_E}{I_M}$$

Values proportional to I_E/I_M obtained in this way are shown plotted against $[BuOH]$ and $[BuOH]^2$ in fig. 16, for various values of i_{295}/i_{340}(exciplex) within the range 0.22 ± 0.15. It can be seen that it is not possible to determine whether the stoicheiometry is in fact 1 : 1 or 1 : 2 with certainty.

Free (unbonded) alcohol concentration was not determined; the opacity of cyclohexane around 3600K makes such determination

Fig. 16

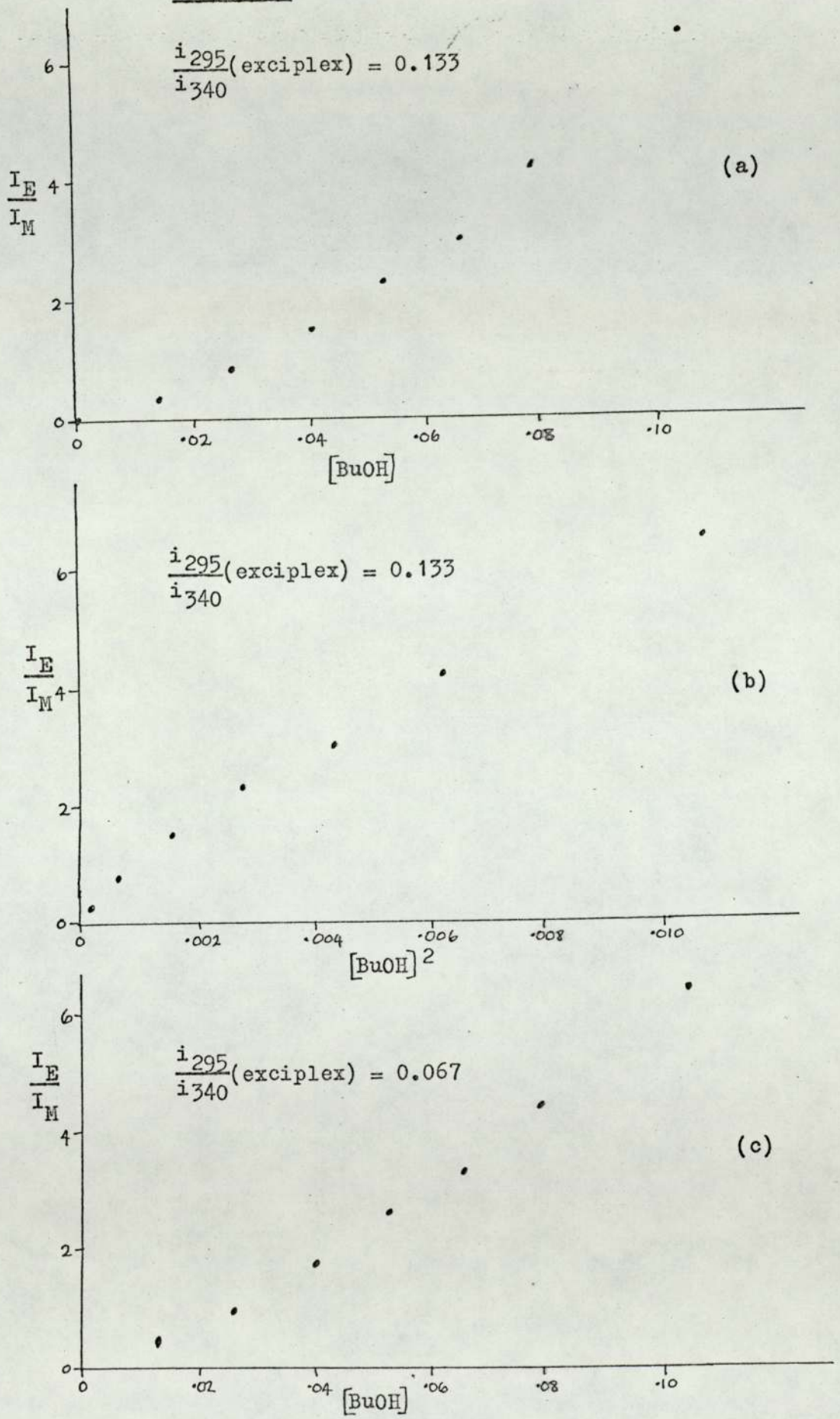
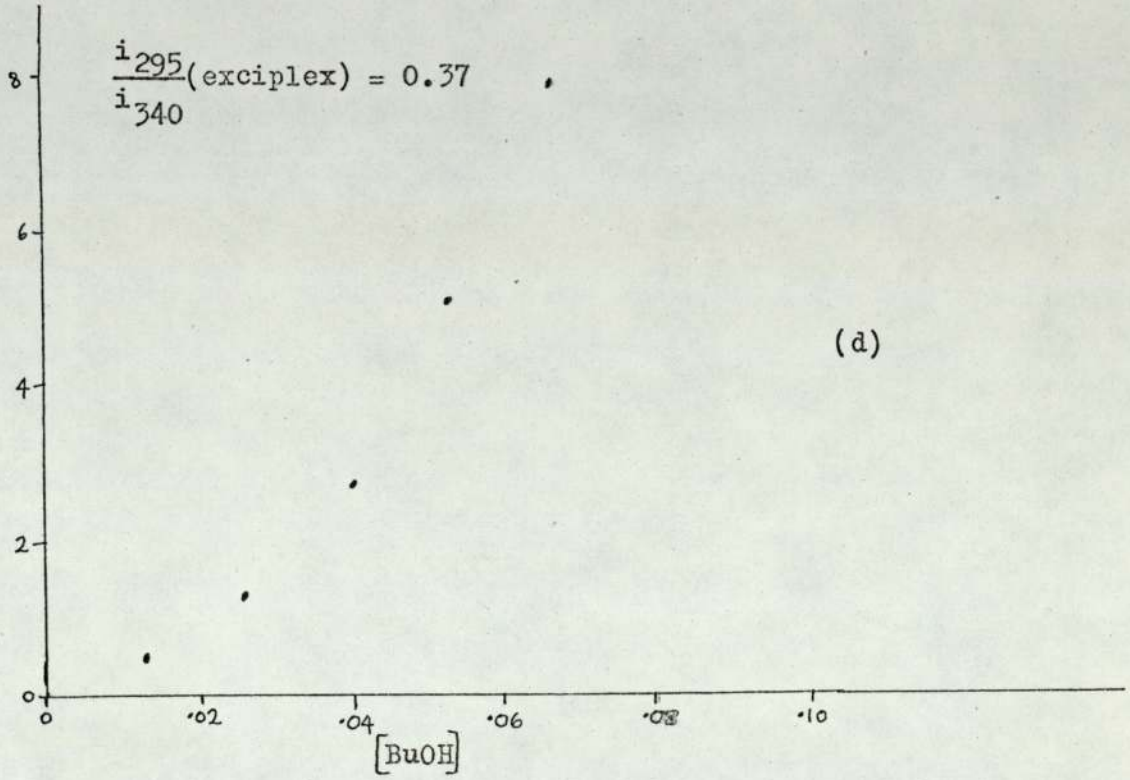
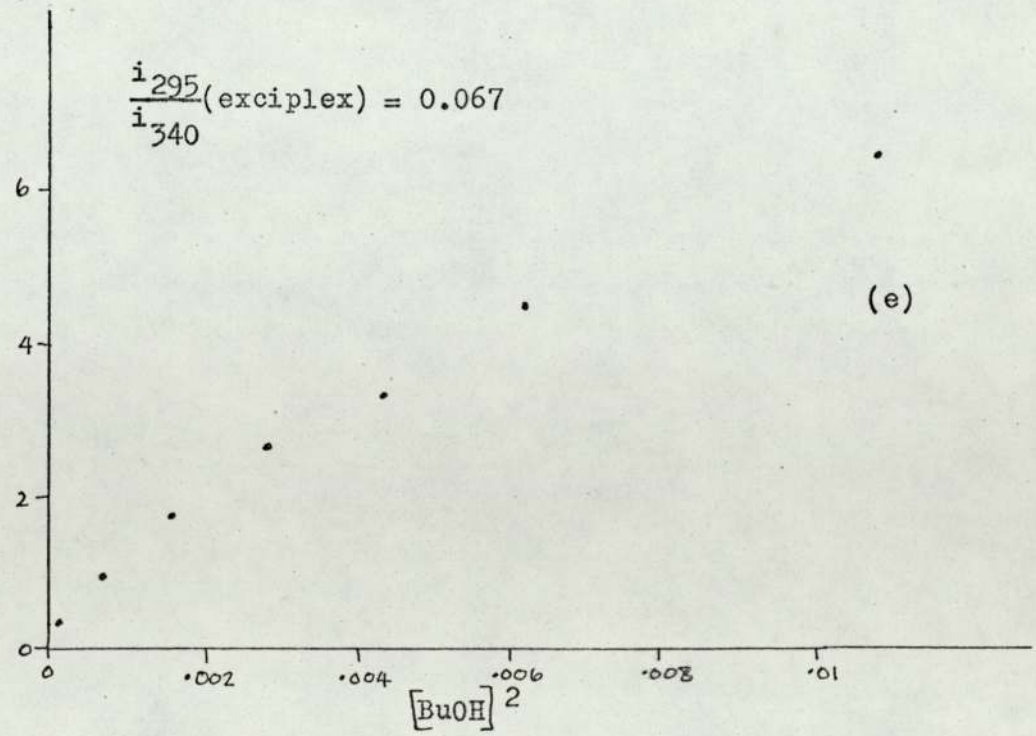

$\frac{i_{295}}{i_{340}}$(exciplex) = 0.133
$\frac{I_E}{I_M}$
(a)
[BuOH]
$\frac{i_{295}}{i_{340}}$(exciplex) = 0.133
$\frac{I_E}{I_M}$
(b)
[BuOH]^2
$\frac{i_{295}}{i_{340}}$(exciplex) = 0.067
$\frac{I_E}{I_M}$
(c)
[BuOH]

Fig. 16 continued



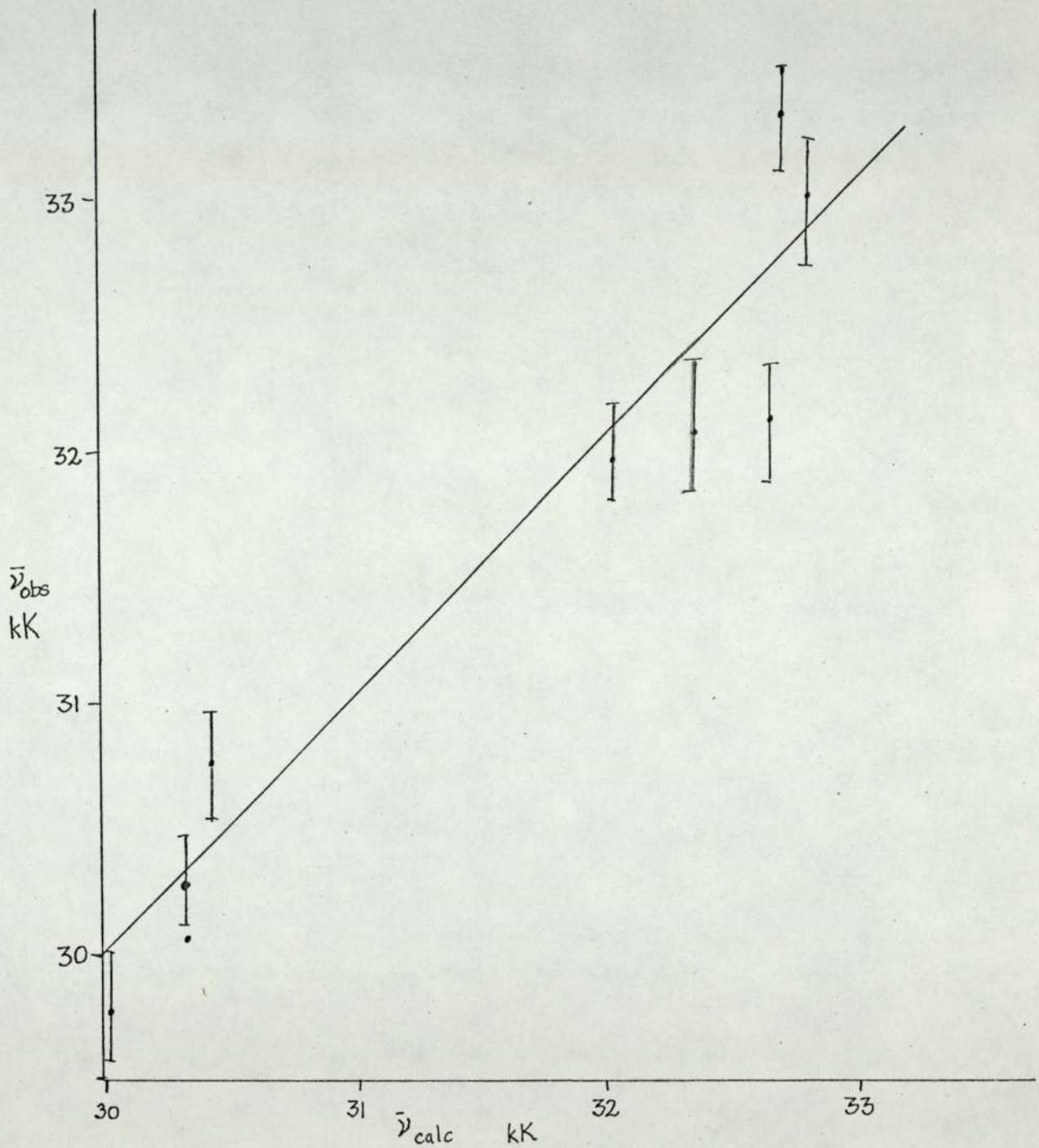


difficult. I.R. results for CCl_4 solutions of alcohols imply that at concentrations below 0.05M, bonded OH concentration is very small[123]. Whilst application of these results to a different solvent system is a precarious process, one would expect that the carbon chain in 1 - butanol would aid solvation in cyclohexane, and hence the approximation [total BuOH] = [free BuOH] will be valid over the concentration range used. In any event, deviations from this approximation would give curvature of the opposite sense to that obtained in fig. 16.

The existence of an isoemissive point seems to indicate that an exciplex is formed; a nonspecific solvent reorientation would not be expected to give this result (ref. 7, p. 286). Presumably if the experimental error were reducible, it would be possible to obtain the stoicheiometry. Perhaps the weight of evidence (Longworth's later result[115] and the iso - emissive point) favours a 1 : 1 stoicheiometry at the present time.

The polar non - hydroxylic solvents do not give such a clear case. Walker et. al.[32] quote pseudostability constants of 0.2 (ether), 0.28 (dioxan), 1.21 (acetomitrile) M^{-1}, compared with 109.6 (1 - butanol) and 141.3 (methanol), for indole. These values imply that the postulated exciplex in the case of the non - hydroxylic solvents is very weakly bound. Furthermore, the spectrum in pure solvent is still structured, consistent with incomplete conversion to the exciplex state. It was decided to investigate further the application of the dielectric formulae with respect to these solvents, in view of

Fig. 17



(Limits represent experimental limits, estimated.)

the fact that the relation tested by Mataga, eq. 24, does not contain the terms in $\frac{D-1}{D+2} - \frac{n^2-1}{n^2+2}$, which are important for cases where both the solute and the solvent are polar[111,112]. Eq. 21 was fitted to the emission maxima observed for indole in eight solvents, by regression analysis using the #XDS3 program supplied by I.C.L. for their 1905E computer. Refractive indices and dielectric constants were obtained from refs. 113 and 116, and the values used are shown in table 4.

Table 4

	Ether	Cyclohexane	Dioxan	EtOAc
obs	33.003	33.333	32.154	32.051
term 1	0.1782	0.2040	0.2026	0.1852
term 2	0.2993	0.0771	0.0316	0.3977
term 3	0.0896	0.0059	0.0010	0.1582

	THF	DMF	DMSO	Formamide
obs	31.949	30.769	30.303	29.762
term 1	0.1969	0.2043	0.2206	0.2112
term 2	0.4358	0.6657	0.6580	0.7054
term 3	0.1899	0.4432	0.4330	0.4976

Values of the coefficients obtained were :-

$(A + B + C) = -8.478$ kK

$E_F = 2.485$ kK

$F_F = -8.677$ kK

and the intercept term was 34.321 kK (291.4nm), which would represent the fluorescence maximum in the vapour spectrum. The correlation coefficient obtained was 0.956, and, as can be seen from fig. 17, the dielectric equation represents adequately the

red shifts found for these solvents. The deviations are slightly larger than those found by Kubota et. al. for pyridine N - oxide (ref. 112, p. 395). However, some deviation would be expected because of the existence of two overlapping bands. These may be differentially shifted by dielectric stabilisation, and if both contribute to the emission, and in different amounts, the observed maximum is neither pure L_a or pure L_b, but somewhere between the two.

It seems, then, that the effect of a non - hydroxylic polar solvent is to associate with the excited indole molecule only very weakly. The complex which may be produced can readily dissociate, and this process occurs during the lifetime of the excited state (in a non - viscous solvent) causing little contribution of the complex to the emission.

C. THE STRUCTURE OF THE EXCIPLEX.

As Walker et. al.[95] pointed out, there is as yet no evidence against the existence of an exciplex of indole with hydroxylic solvents. Neither has there been, however, much discussion in the literature as to the structure of the complex, which from the stoicheiometry must be a sterically definable entity.

On excitation of the indole molecule, an electronic transition occurs from one orbital to another. This alters the charge distribution around the molecule, and since the energy increases, the redistribution of charge involves charge separation. In other words, the resonance hybrid contains a higher contribution of ionic forms in the excited state than in the ground state, Nevertheless the molecule is still stabilised by delocalisation, and interactions which disturb this stabilisation (e.g. electrophilic attack at C2) will be to this extent unfavoured in the excited state as in the ground state. The increase in electron density which occurs at C2 relative to C3 (the usual position of attack in the ground state) will offset this, perhaps to the extent that C2 may become the preferred position for interaction in the excited state.

A number of studies of ground state complexes of indole have been reported, and it is perhaps pertinent to review some of these, since they may provide a starting point for the understanding of excited state complexes.

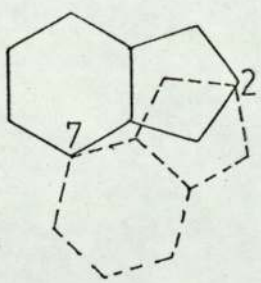
A recent publication of Cazeau - Dubroca et. al.[117] showed from the temperature dependence of the absorption spectra of

indole and N - methylindole that there are hydrogen bond interactions between indole and solvent in propyl ether. In 3 - methylpentane, indole was observed to self - associate by hydrogen bonding, but this occurred only at low temperatures.

The formation of exciplexes of N - methylindole with hydroxylic solvent precludes the possibility of hydrogen bonding of this type, via the indole N-H, in the exciplex.

Reinecke et. al.[118] examined the dilution shifts of proton resonances in the N.M.R. spectra of indoles. The greatest shifts were found for protons in the 2 and 7 positions. The authors interpreted their results in terms of a dimer, probably of the type shown in fig. 18.

Fig. 18



The interaction was not seen as a charge transfer type, where the 3 position is most sensitive to added acceptors.

Optical spectroscopic measurements led Szent - Gyorgyi[119] to conclude that iodine complexes with indole in a localised manner over the 2 - 3 positions. Foster and Fyfe[120] supported this conclusion for other ligands (trinitrobenzene, dinitro - benzene) by N.M.R. experiments. They found that addition of

trinitrobenzene to dilute indole solution caused the whole of the indole spectrum to shift, but the proton resonance at C3 shifted more than the other absorptions.

Molecular orbital calculations on donor - acceptor complexes of indole by Green and Malrieu[121] showed no correlation of the total charge on C3 with donor capacity, however there was some correlation of the superdelocalisability for electrophilic reaction at C3 with the donor capacity.

It may be concluded that two types of association occur, a less specific interaction involving only dipolar attractive forces, and a localised interaction involving the C3 position acting as donor in a DA type of complexation.

In rejecting the first of these as a possible exciplex candidate, the pseudostability constants of Walker's exciplexes (K)[32] may be quoted. These were based on the kinetic scheme:

$$\begin{array}{ccc} A^* + nS & \underset{k_2}{\overset{k_1}{\rightleftharpoons}} & AS_n^* \\ \downarrow k_3 + k_4 & & \downarrow k_5 + k_6 \\ A & & A \end{array}$$

(where k_4, k_5 are radiative, k_3, k_6 nonradiative constants)

$$\text{and} \qquad K = \frac{k_5 k_1}{k_4(k_2 + k_5 + k_6)}$$

In a non - viscous solvent, k_1, k_2 would be expected to be ca. 10^{11} to 10^{13}, whereas k_3, k_4, k_5, k_6 are ca. 10^9.

Therefore,

$$K \doteqdot \frac{k_5 k_1}{k_4 k_2}$$

and if radiative rate constants are similar for A^* and AS_n^*,

$$K \doteq \frac{k_1}{k_2}$$

i.e. K is approximately the equilibrium constant for exciplex formation.

The values obtained by Walker et. al. were: (with 1 - butanol)

indole	109.6
1 - methylindole	12.03
1,3 dimethylindole	44.57

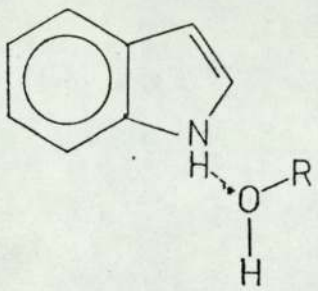
It can be seen that the 1,3 - dimethylindole exciplex is stronger than that of 1 - methylindole. If a less specific interaction was involved, then steric effects would be expected to be important, and the above observation is not consistent with this hypothesis.

One type of DA complex is the hydrogen bonded complex. Since all the solvents under consideration here are hydroxylic, it is possible that they could interact with indole in the excited state by forming a hydrogen bond to the indole ring, the indole (perhaps at C3) donating electrons to the lowest energy antibonding orbital of the O-H bond. For this to be valid, no such interaction should occur in the ground state. Consequently, an I.R. study of hydrogen bonding in the ground state was undertaken.

Ground state Hydrogen bonded Complexes. - Of the solvents cyclohexane, n - hexane, 2 - methylpentane and isooctane, the last mentioned was found to be most transparent in the 3600K region of the I.R. spectrum, and was used in this study. The free O-H peak of ethanol (0.025M dissolved in isooctane)

was observed in the absence and presence of 0.025M indole. No significant change in this peak occurred, the only difference being the appearance of the indole N-H peak (fig. 19). If ground state hydrogen bonding occurs, then, it is in such low amounts as to be undetectable in solutions of concentration below 0.025M.

Consider the mode of chemical interaction of an alcohol with indole. The indole may act as proton donor:



However, substitution of the N - hydrogen by methyl does not alter the capacity to form exciplexes. Thus if this bonding does occur, it is of minor significance in the present context.

Alternatively, the indole ring may act as proton acceptor:

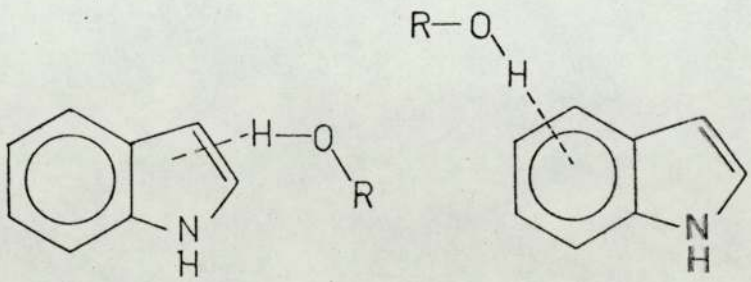
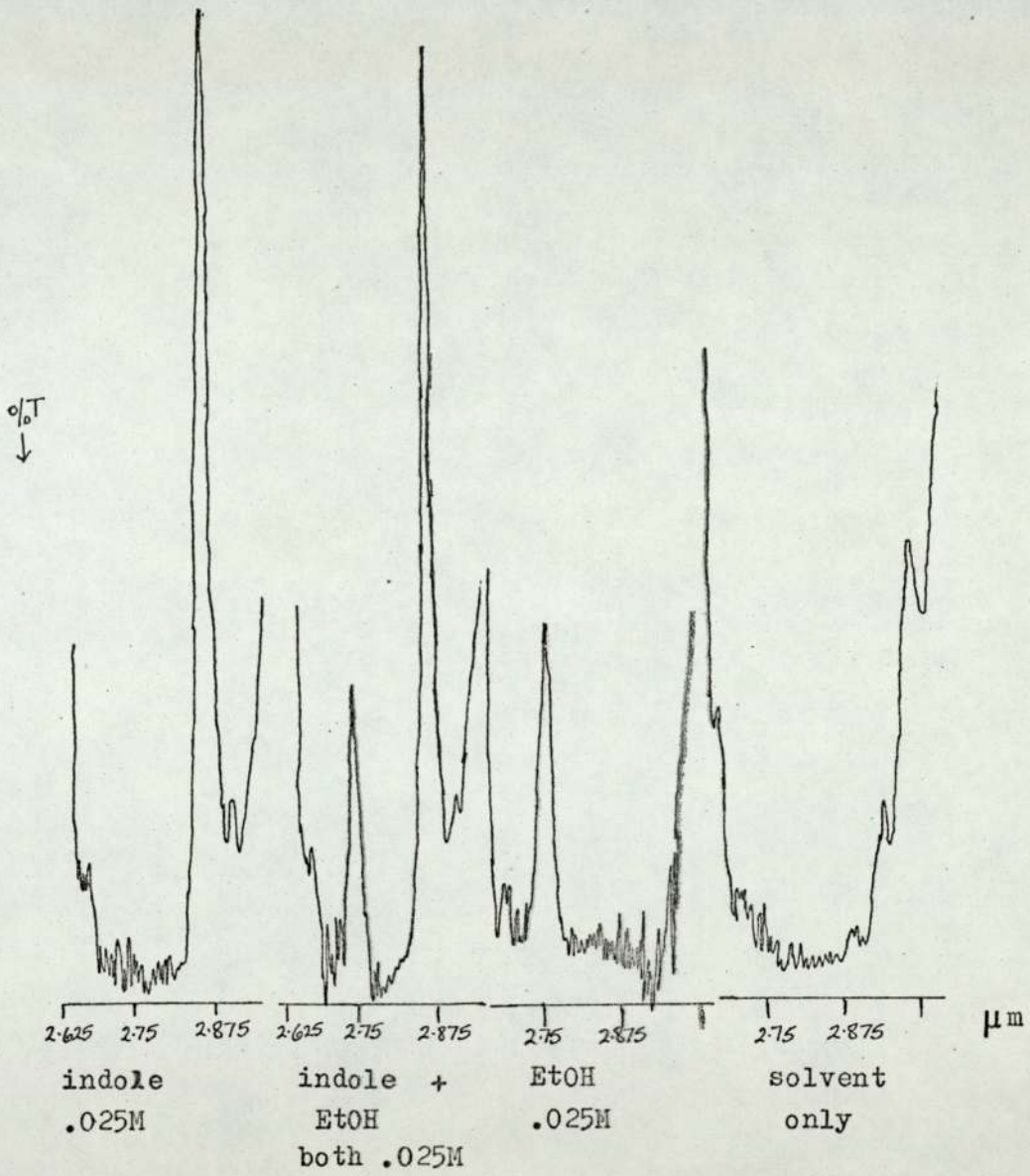
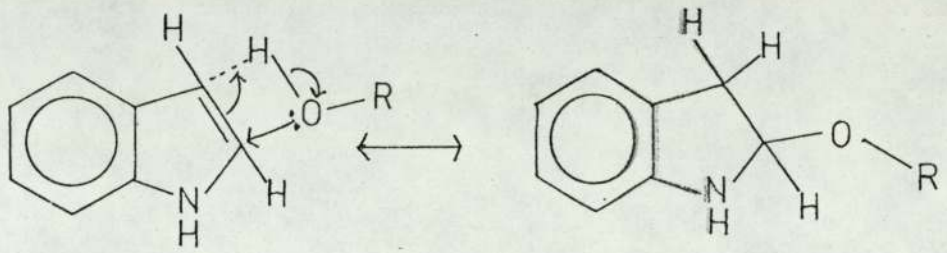


Fig. 19


%T
2·625
2·75
2·875
2·625
2·75
2·875
2·75
2·875
2·75
2·875
μm
indole .025M
indole + EtOH both .025M
EtOH .025M
solvent only

Such interaction would presumably be localised. The electron densities in the S_1 state as predicted by molecular orbital calculations are high at N1, C2 and C3 of the pyrrole ring, and C4 and C7 in the benzene ring. If localised interaction with the pyrrole ring is considered, the oxygen may also participate:



An exciplex based on an interaction of this type would be visualised as possessing an electronic structure similar to the transition state for the above reaction.

Such an interaction may be expected to give rise to photo - products (this explaining the reduction in fluorescence quantum yield upon exciplex formation). A preliminary irradiation of an indole solution gave products with an absorption spectrum not characteristic of either an indole or an indoline (but more like an oxindole). This line of investigation was not pursued further.

In search of further evidence for this indoline model, the fluorescence of indoline was examined, and its emission spectrum is presented in fig. 20. A similarity is immediately noted

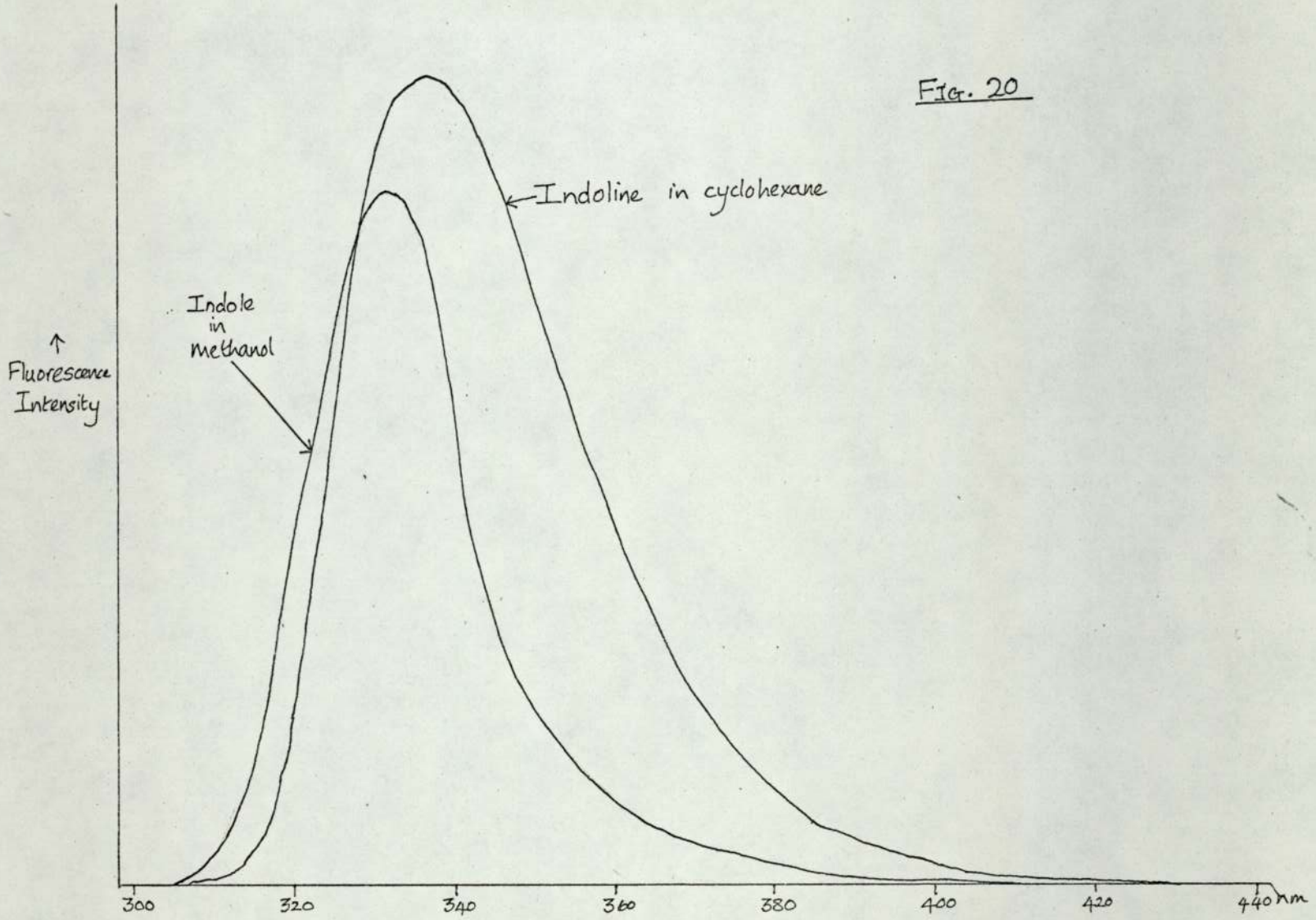


Fig. 20
Indoline in cyclohexane
Indole in methanol
Fluorescence Intensity
300
320
340
360
380
400
420
440 nm

between the spectrum of indoline in cyclohexane and the spectrum of the indole - methanol exciplex. A further similarity is shown in the lifetimes of these compounds:

Table 5

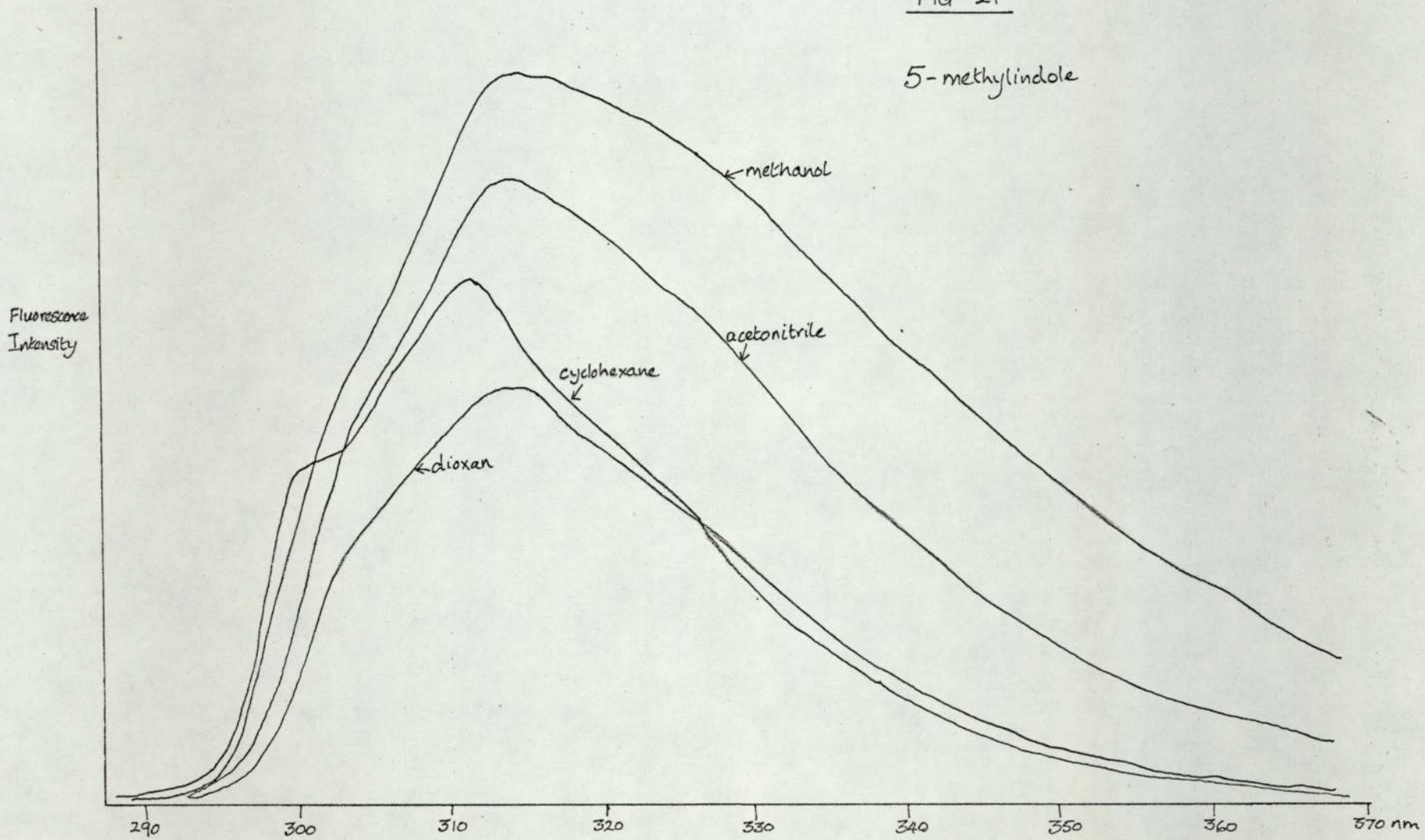
compound	measured lifetime (ns)
Indole (cyclohexane)	9.0 (lit. 9.1[30], 7.8[29])
Indole (methanol)	4.3 (lit. 4.1[29])
Indoline (cyclohexane)	2.2

On this (slender) evidence, then, indoline appears to be a suitable model for the exciplex of indole.

Recalling now the hypothesis of Mataga et. al.[31], in which the authors claimed stabilisation of the ^1L_a state to a greater degree than the ^1L_b state by interaction with polar solvent, leading to emission from the ^1L_a state in such solvents, it is noted that the reduction in lifetime on going from cyclohexane to methanol is in accordance with this hypothesis. It was pointed out earlier in this chapter that the $^1L_a - {}^1L_b$ separation in 5 - methylindole is much greater than in indole itself (in cyclohexane). Consequently, the fluorescence of this compound was also examined, to find whether state reversal could explain the emission in this case also. The spectra of 5 - methylindole in various solvents are presented in fig. 21, and in fact, a large red shift was not observed. This leads to the following possibilities:

i) the emission is from the ^1L_a state in hydroxylic solvents, interaction causing state reversal as postulated by Mataga.

FIG 21


5-methylindole
Fluorescence Intensity
methanol
acetonitrile
cyclohexane
dioxan
290
300
310
320
330
340
350
360
370 nm

Exciplex formation is only a weak perturbation on the energy levels as stabilised by dipole - dipole interaction;

ii) 5 - methylindole does not form an exciplex with hydroxylic solvents.

Possibility (ii) was discarded after a fluorometric titration of 5 - methylindole in cyclohexane with 1 - butanol revealed that spectral alterations were produced in 0.2M butanol (fig. 22). Wavelength shifts were of such a small magnitude that quantitative experiments were not possible with our apparatus. Nevertheless, it was concluded that exciplexes are formed between 5 - methylindole and hydroxylic solvents. The emitting level in 5 - methylindole is ^1L_b, in both non - polar and polar solvents.

The lifetime of 5 - methylindole was also measured (table 6)

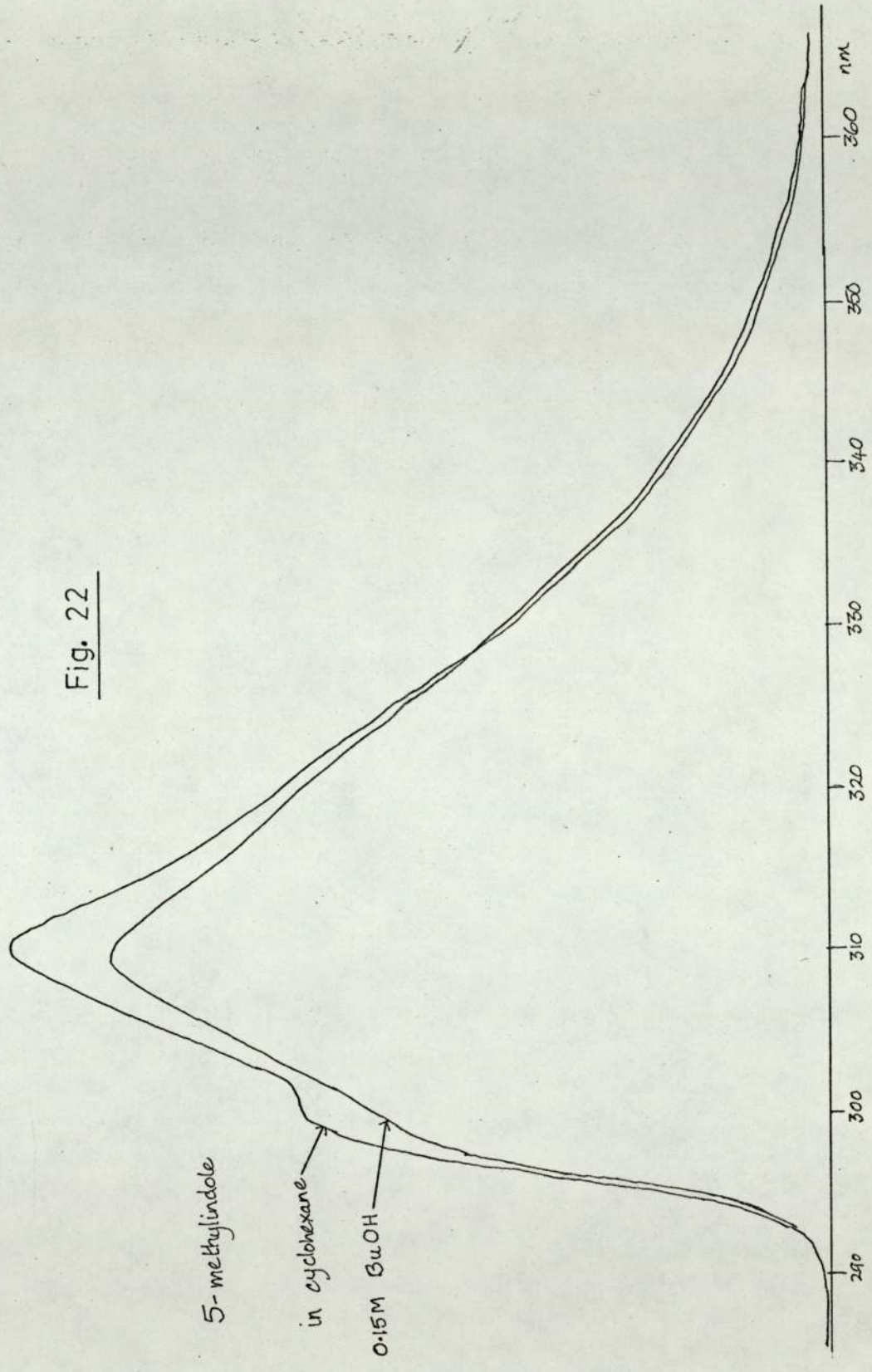
Table 6 Lifetimes of 5 - methylindole

Solvent	Measured lifetime (ns)
Cyclohexane	9.7
Cyclohexane + 1 drop BuOH	9.0
Methanol	4.9

Conclusions

It seems likely that in hydroxylic solvents, a hydrogen bond is formed between the excited indole molecule and a solvent molecule. This interaction does not in itself cause the observed red shift, but may add to the dipole - dipole interaction which preferentially stabilises the ^1L_a level. This stabilisation is sufficient to place the ^1L_a level of indole and 3 - methylindole below the ^1L_b level during the excited

Fig. 22


5-methylindole
in cyclohexane
0·15M BuOH
290
300
310
320
330
340
350
360
nm

state lifetime, but is insufficient to reverse the levels of 5 - methylindole. A lifetime of ca. 9 ns is characteristic of emission from the ^1L_b level of indoles, and 4 ns from the ^1L_a in cyclohexane, but these values are altered in methanol, and a lifetime of ca. 4 ns in methanol is not necessarily indicative of emission from the ^1L_a state.

INTERACTION OF EXCITED INDOLE WITH THE CARBONYL GROUP

Introduction.

Mechanisms for the quenching of indole fluorescence by a side chain carbonyl group already discussed in the literature were mentioned in the first chapter, and can be summarised as follows:-

i) electron scavenging by the carbonyl group[38];

ii) intramolecular enhancement of vibrational coupling between the excited and ground states[8];

iii) electrostatic interaction leading to an excited state charge transfer complex[39].

With the compounds examined by Cowgill[3,8], a polar solvent was also a requirement for quenching to occur. This requirement may be interpreted as either

i) the indole - solvent exciplex is quenched by the carbonyl group (and not the un - exciplexed indole) , or

ii) the carbonyl group itself is not a potent quencher, but the solvated carbonyl group causes quenching.

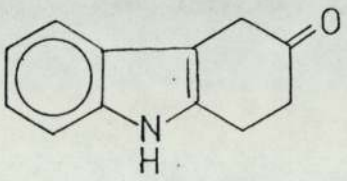
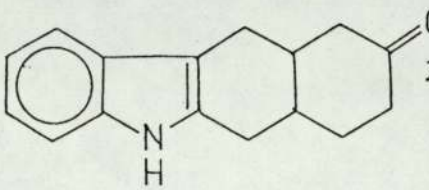
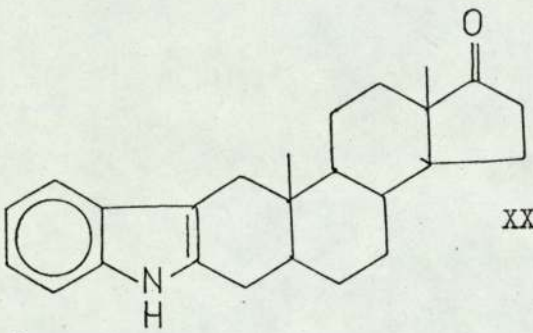
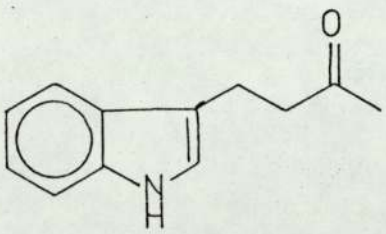
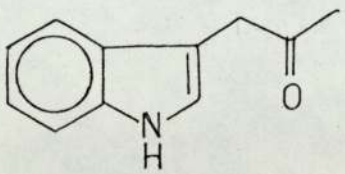
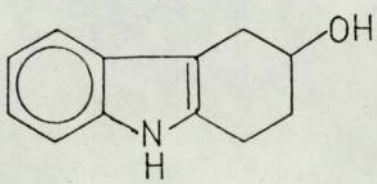
In fact, Cowgill's results[3] show that as organic solvent is added to an aqueous solution of N - acetyltryptophan methyl ester (of low quantum yield), the quantum yield rises continuously with the increase of mole fraction of organic solvent. If case (i) above was to be the operative explanation, then maximal quenching would be expected with only a small amount of water present in the organic solvent. It appears, therefore, that indole fluorescence is quenched by a solvated carbonyl group (but see later arguments in this chapter).

In view of the low number of compounds forming the basis of these conclusions, and to try to interpret the results in a more detailed manner, the syntheses outlined in chapter 2 were undertaken. The results showed that the carbonyl groups could be divided into two types, viz. ketonic and non - ketonic. This division may be justified on the basis that the ketone group absorbs maximally around 280nm, (the $n-\pi^*$ absorption), but the long wavelength edge of this absorption overlaps the emission spectrum of indole, making excitation energy transfer possible. Thus, any carbonyl which has this overlap can be classified along with the ketones. The other types examined here, i.e. acid, ester, amide - do not have this absorption, and energy transfer is insignificant. The results are now considered in terms of this classification.

Indole Ketones.

Quantum yields are presented in table 1. Fluorescence from indol - 3 -yl acetone was undetectable by the Aminco fluorimeter, but repeated recrystallisations of this compound did not remove its buff colouration. It is possible that it exists in the enol form, in which case the chromophore is non - indolic. This compound will therefore be eliminated from further discussion. Enolisation could also occur in compound XIII. However, addition of acid or alkali to a methanolic solution of this ketone did not alter its U.V. absorption spectrum, indicating that enolisation did not significantly occur under these conditions. XIII is therefore considered

Table 1 Quantum yields of indole ketones

Compound		Q(cyclohexane)	Q(MeOH)
	XIII	0.003*	0.040
	XV	0.0066*	0.075
	XXII a	0.029	0.20
	b		0.23
			0.002*
			0
cf. 		0.38	0.31

* = error not determined; others have estimated accuracy of ± 6%

along with the other ketones.

Consideration of the cyclic ketones shows that as the carbonyl group is moved further from the chromophoric nucleus, the quantum yield increases. Using molecular models, the distance of the carbonyl from the indole 2 - 3 bond was estimated, and the quenching is shown in fig. 1 plotted against the reciprocal of this distance. (table 2)

Table 2

Compound	Indole - CO distance (R, 10^{-10}m)	$\frac{Q(\text{ref. OH cpd.})}{Q(\text{CO cpd.})}$	$\frac{1}{R}$
XIII	1.4 - 2.9	7.7	0.71 - 0.34
XV	3.5 - 5.4	4.1	0.29 - 0.19
XXIIa	6.5 - 9.0	1.5	0.15 - 0.11

It is seen that there is an approximate 1/R dependence of the quenching. This is consistent with energy transfer from indole to carbonyl by the exchange mechanism[124,125], which may be expected for groups as close as less than 1nm. Forster's mechanism[125], which accounts for transfer between groups separated by e.g. 5nm, requires a $1/R^6$ dependence of the quenching, which is clearly not the case with these ketones.

Indolylbutanone is very efficiently quenched, and this may be ascribed to the freedom of the carbonyl group to orient itself, allowing more efficient energy transfer to occur.

The fluorescence spectra of the indole ketones resemble those of the control compounds (either tetrahydrocarbazole or its 3 - hydroxy derivative), being reduced in intensity, but not positionally altered. An exception to this is compound

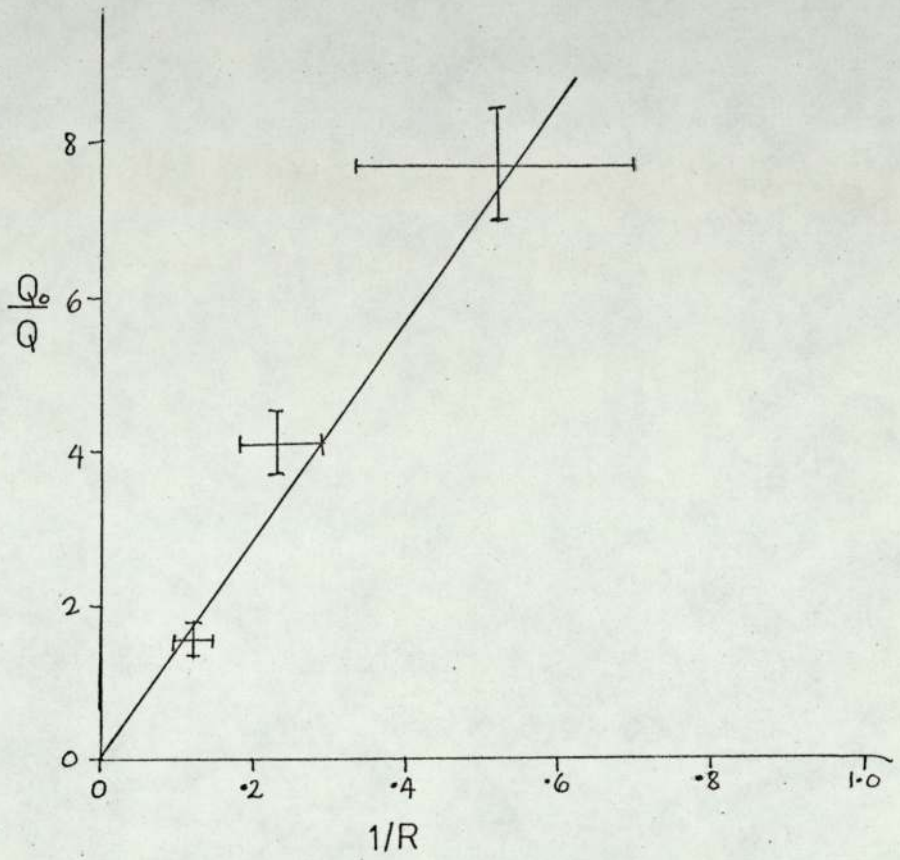


Fig. 1

XIII in cyclohexane, whose spectrum is shown in fig. 2. The emission of this compound is at a longer wavelength, 370nm, than that of the parent compound. This may be explained by the low quantum yield. There is sufficient energy transferred to the ketone to elicit measurable emission from this group. Cyclohexanone itself is fluorescent, emitting maximally at 405nm with a quantum yield of 1.7×10^{-3} (126). Fig. 3 shows how the observed spectrum may be accounted for by a sum of emissions from the indole and ketone moieties. Emission from the carbonyl group in the other ketones would be masked by the more intense indolic emission.

Fig. 2

Fluorescence spectrum of XIII in cyclohexane.

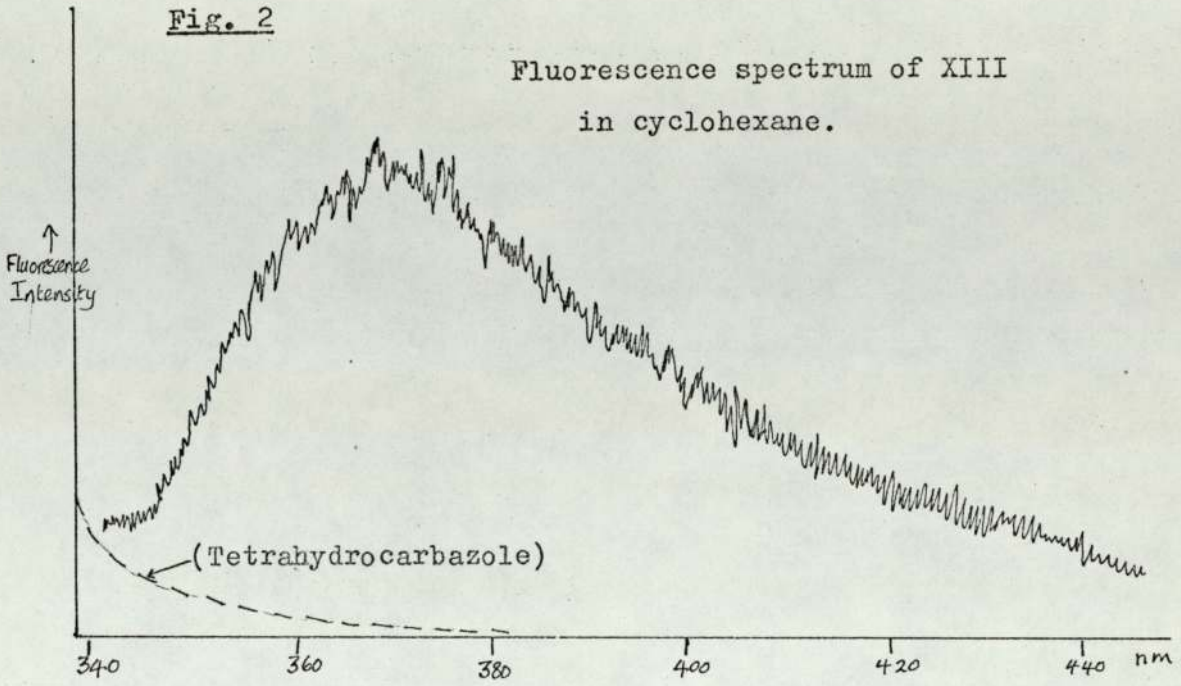
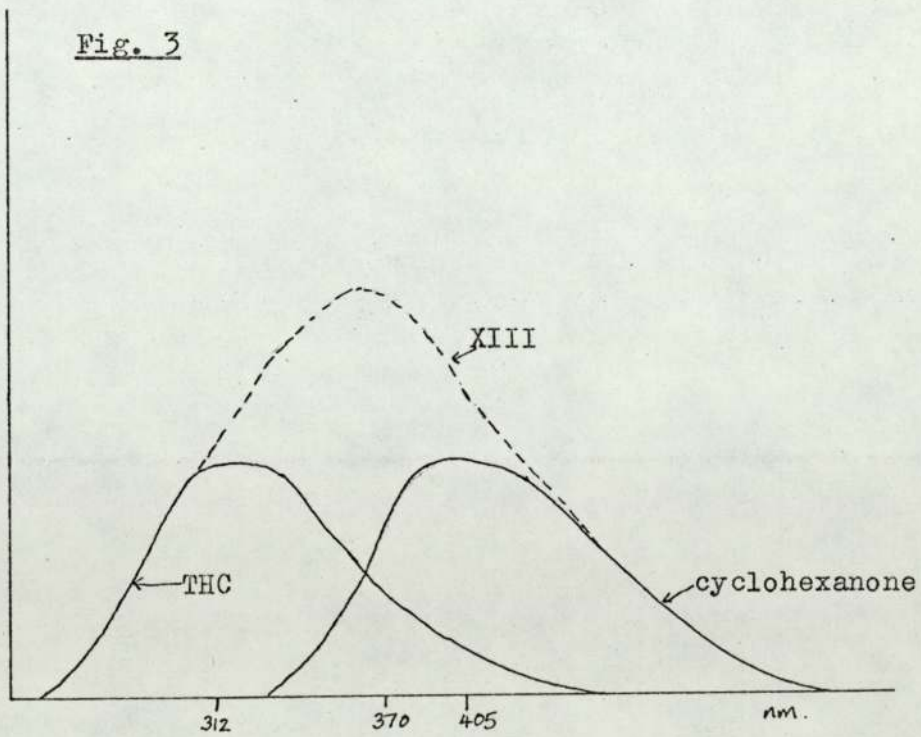


Fig. 3



Non - Ketonic Indole Carbonyl compounds.

The quantum yields of various carbonyl compounds and their non - carbonyl analogues are presented in table 3.

Table 3 Quantum yields (Q), based on p - terphenyl = 0.77[131] in cyclohexane. Literature values in parentheses.

Compound	Q(cyclohexane)	Q(methanol)
Indole	0.55 (0.59[30])	0.28(0.30[29])
3 - substituted indoles		
3 - methylindole	0.40	0.32_5
3 - ethylindole	0.40	
3 - indoleacetic acid		0.19
3 - indolepropanoic acid	0.32*	0.29
3 - indolebutanoic acid	0.23*	0.30
3 - indoleoctanoic acid		0.29
3 - indoleacetate anion		0.23*
3 - indolepropanoate anion		0.29*
3 - indolebutanoate anion		0.30*
3 - indoleacetamide	0.23	0.23
Ethyl 3 - indoleacetate	0.47/9%	0.12
Methyl 3 - indolepropanoate	0.41	0.23
Methyl 3 - indolebutanoate		0.24
3 - hydroxymethyleneindole	0.43	
3 - (2' - hydroxyethyl)indole	0.41	(0.33[39])
L - Tryptophan	0.13	0.08_6
N - Acetyl - L - tryptophanamide		0.17
DL - Tryptophanol		0.30
Alanyltryptophan		0.047
L - Tryptophan anion		0.26*
2,3 - disubstituted indoles		
2 - Methylindol - 3 - ylacetic acid		0.18
2 - Methylindol - 3 - ylpropanoic acid		0.26
2 - Methylindol - 3 - ylacetate anion		0.22*

Table 3 continued.

Tetrahydrocarbazole derivatives (THC)		
THC	0.34/6%	0.32
3 - hydroxy THC	0.38	0.31
3 - acetoxy THC		0.35
3 - oxo THC ethylene ketal		0.31
THC 3 - carboxylic acid		0.31
Methyl THC 3 - carboxylate		0.29
3 - Amino - 3 - carboxy THC		0.32_5
3 - Acetamido - 3 - carboxy THC	0.23*	0.31
Tetrahydrocarbolines		
L - tetrahydrocarboline-3-carboxylic acid		0.31
Yohimbinic acid		0.38
Miscellaneous		
Cyclopent -[b]- indole		0.33
Cyclohept -[b]- indole		0.38
Indoline	0.18*	
		0.34/7%

* Results based on a single measurement, the error being undetermined. Other results were from multiple determinations, and the error is estimated to be within ± 6%, except where otherwise stated.

It can be seen from this table that in general, compounds which are quenched in methanol are not quenched to the same degree in cyclohexane. The exceptions to this are tryptophan and indoleacetamide. Both of these compounds are insufficiently soluble in cyclohexane to permit accurate measurements to be made, and the error is likely to be high. The presence of a contaminant of high solubility in cyclohexane may have quenched

the fluorescence, although no abnormality in the absorption spectra was noticed. Were the contaminant itself indolic, the fluorescence should not have been quenched. Thus, Cowgill's requirement of a polar solvent is borne out partially by the results obtained here.

Correlation with separation of indole and carbonyl groups. - Several series of compounds are presented in table 3. Consideration of the yields of the indolealkanoic acids shows that as the chain length is increased, the quantum yield rises; a similar effect is seen with the esters of these acids. This supports a collisional mechanism of quenching.

Orientation of the carbonyl group. - All the derivatives of tetrahydrocarbazole have quantum yields close to tetrahydro - carbazole itself. This indicates that the carbonyl group must approach the indole nucleus either closer than the tetrahydro - carbazole ring system will allow, or in some particular orientation. This favours a specific interaction type of mechanism.

Carbonyl type. - In order to obtain more information about this mechanism, the effect of varying the carbonyl group at a particular position was investigated. One means of doing this was to ionise the acids and amino acids. Fluorescence titration curves for several of these compounds were obtained and these are shown in figs. 4 and 5. The results show that the change in fluorescence is associated with the ionisation of the side chain (pK values are given in table 4). This agrees with the results of Cowgill[3].

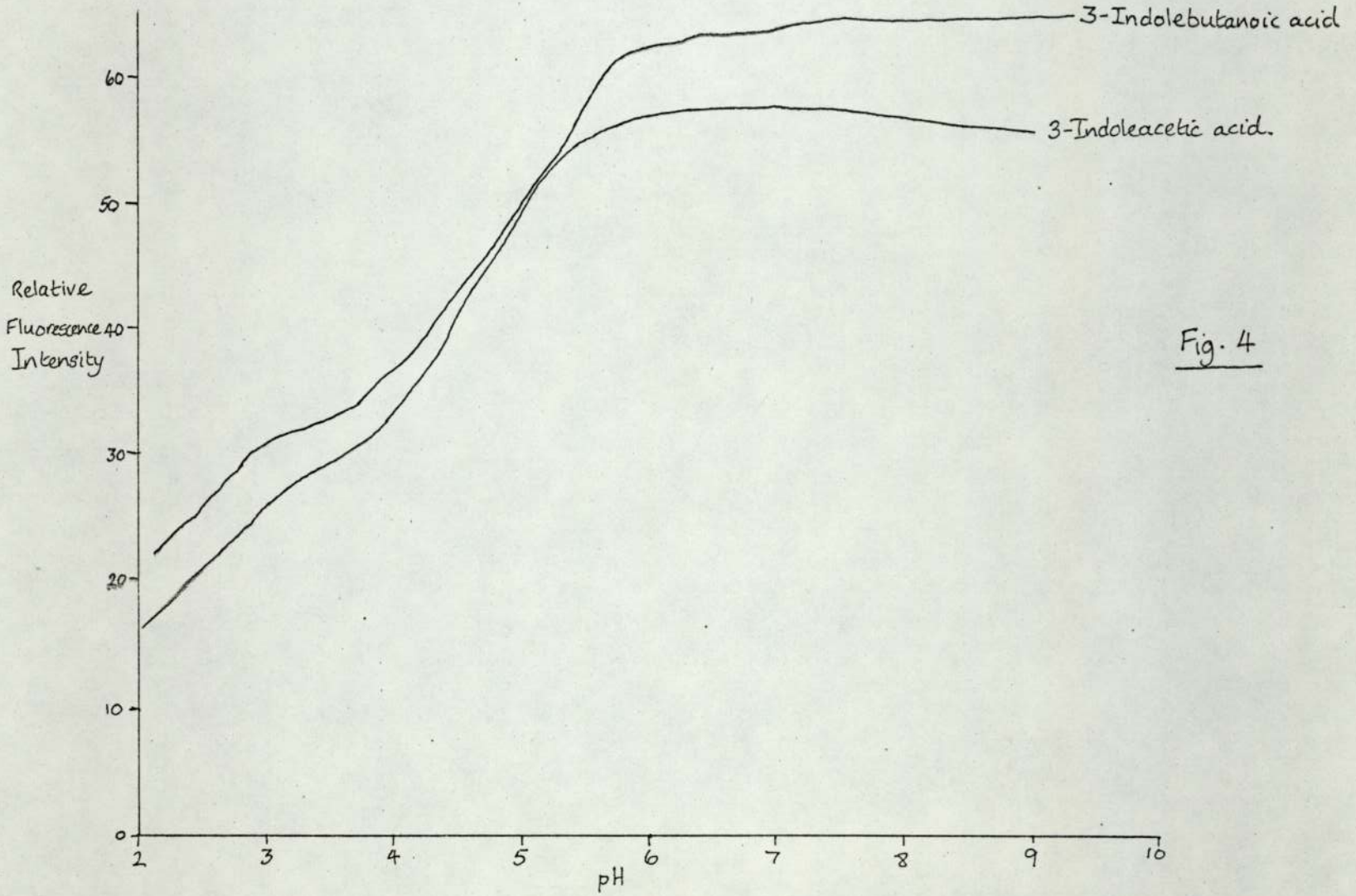

3-Indolebutanoic acid
3-Indoleacetic acid.
Relative Fluorescence Intensity
60
50
40
30
20
10
0
2
3
4
5
6
7
8
9
10
pH

Fig. 4

Fig. 5

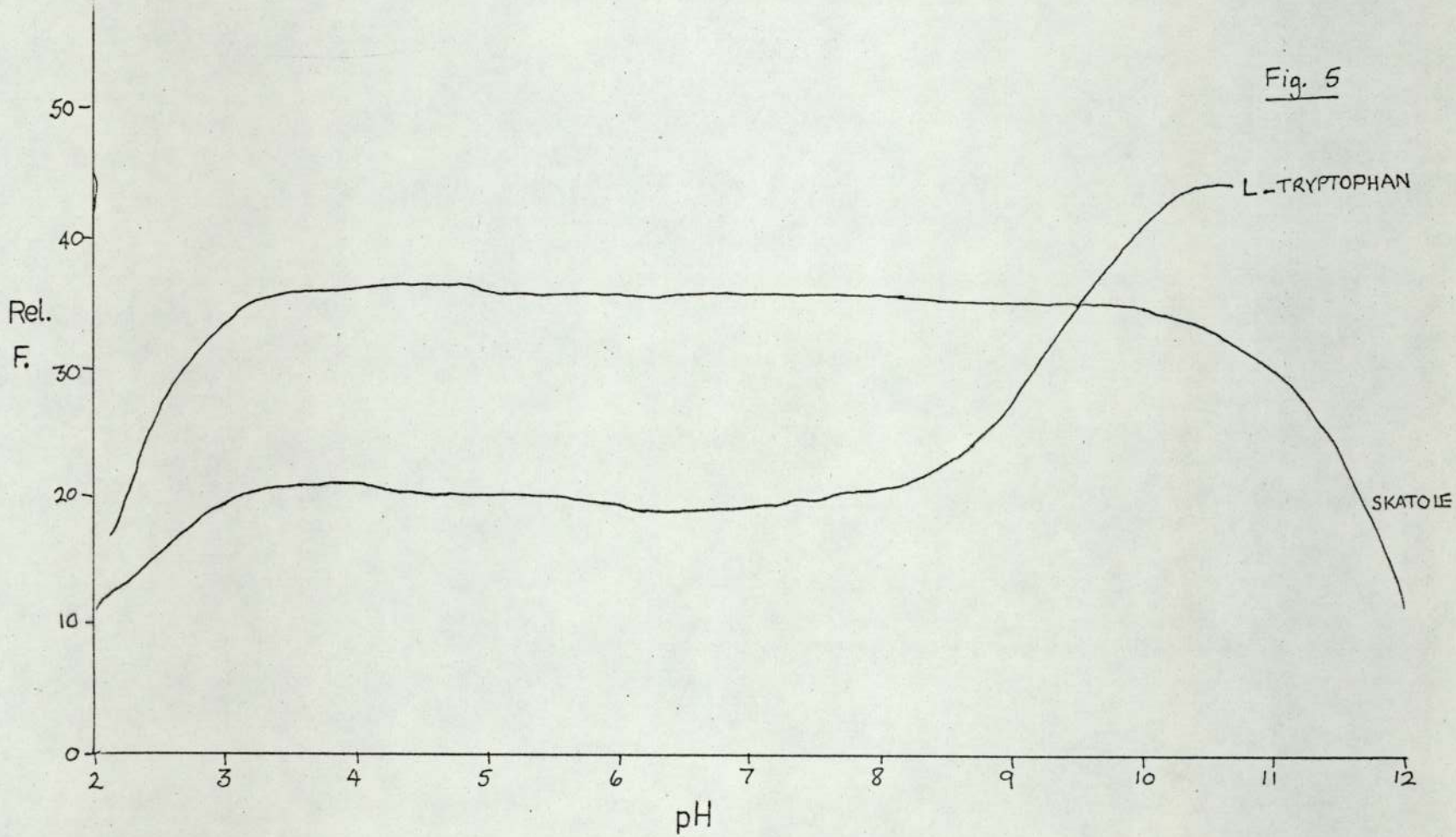


Table 4 pK values

3 - indoleacetic acid	4.66 ± 0.05
3 - indolebutanoic acid	5.18 ± 0.05
Tryptophan	Lit. 2.38, 9.39[129]

It is interesting to note that the titration curve e.g. of indolebutanoic acid, shows that as the carboxylate group becomes protonated, the quantum yield decreases by a factor of ca. 2. The quantum yields determined with methanol as solvent are 0.30 for the acid, with no change on adding a drop of methoxide solution. This implies that these acids are fully ionised in methanolic solution at concentrations around 10^{-5}M. This was verified by conductance measurements in the case of indolebutanoic acid, which showed that at 5×10^{-4}M, the degree of ionisation was ca. 0.75, becoming unity at concentrations less than 1×10^{-4}M in absolute methanol.

From the titration curves and the values in table 3, the yields of the unionised acids may now be calculated. (table 5). Having obtained the yields of various acids and their anions, these may be considered along with other derivatives, the esters and amides. One may examine series in which the carbonyl groups are separated by a constant number of carbon atoms from the indole ring, but are different in type. In this respect, one series of compounds was considered in detail, namely the indoleacetic series. This was chosen because of the greater variations shown by this series than by its homologues.

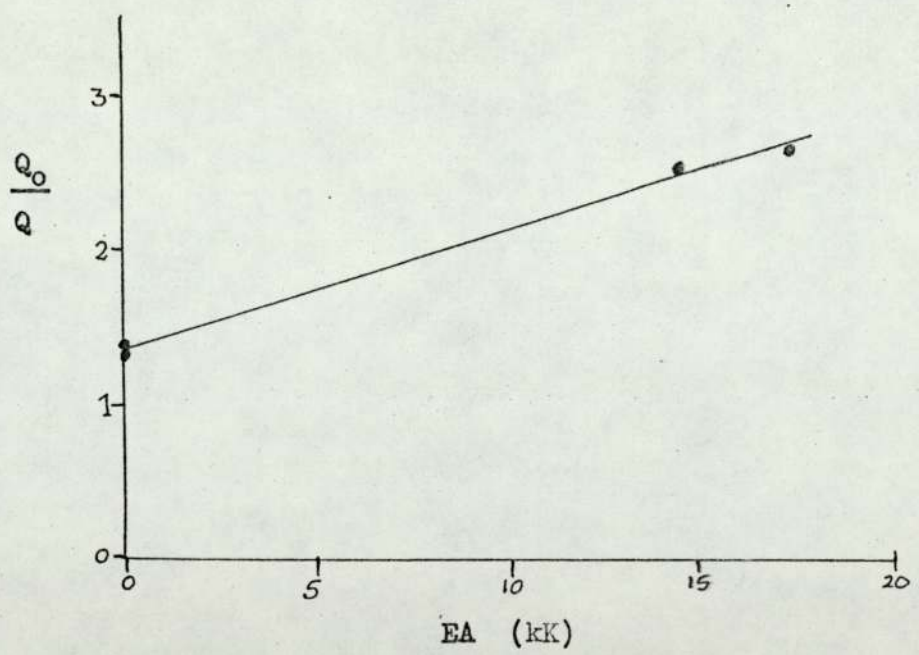
Indoleacetic acid derivatives. - The mechanisms for quenching alluded to at the beginning of this chapter imply that if charge transfer is involved, it will be with the excited indole

Table 5

Quantum yields of unionised acids in methanol

3 - indoleacetic acid	0.13
3 - indolepropanoic acid	0.15
3 - indolebutanoic acid	0.19

Fig. 6



acting as donor, the quencher as acceptor. It may be expected, therefore, that the quenching ability of a particular group will be related to its ability to accept electrons, i.e. to its electron affinity (EA). In order to test this hypothesis, the EAs of the carbonyl groups were required. These were obtained by measurements on compounds representing the side chains, i.e. acetic acid itself, and its derivatives. In view of the quenching requirement of a polar solvent, measurements were performed using the same solvent as for the quantum yields, i.e. methanol. Two methods were used:

i) The charge transfer absorption band of iodide ion - solvent depends on the EA of the solvent (ref. 112, p. 208):-

$$h\tilde{\nu}_{CT} = W_{exc} - W_{gnd}$$
$$= IP_{donor} - EA_{acceptor} - \text{constant} \qquad (1)$$

where W's are energy levels, and IP = ionisation potential.

Tetrabutylammonium iodide was found to have a maximum of absorption at 221nm in methanol. On addition of ethyl acetate, this band decreased in intensity and a new band at 357nm appeared (which was shifted to 363nm in pure ethyl acetate). Of the acetic acid derivatives tested, ethyl acetate gave the largest CT shift, and ethyl indoleacetate was the least fluorescent of the indoleacetic series.

Since the donor is the same throughout the series, IP_{donor} of eq. 1 may be absorbed into the constant term. If values are calculated relative to a scale in which the EA of methanol is taken as zero, then the constant becomes 45.25kK (=221nm), i.e.

$$EA = 45.25 - h\tilde{\nu}_{CT} \qquad (2)$$

The observed frequency shifts of tetrabutylammonium iodide

gave the following EA values for a number of acetyl compounds:

Table 6 Electron Affinities by iodide CT absorption

Compound	EA (kK)
Ethyl acetate (pure)	17.70
Ethyl acetate (in methanol)	17.24
Acetic acid (glacial)	17.51
Acetic acid (in methanol)	14.58
Acetone	18.00
Acetamide (in methanol)	(0)
Sodium acetate (in methanol)	(0)

Dissolving acetamide or sodium acetate in methanol produced no shift of the CT band.

ii) The polarographic half wave reduction potential of a compound is given by the equation[127]

$$E_{\frac{1}{2}} = EA + \triangle F_{solv} + K \qquad (3)$$

where $\triangle F_{solv}$ is the heat of solvation associated with the reduction and K is a constant dependent on the reference electrode and the solvent[128].

Half wave potentials were determined with difficulty for the compounds shown in table 7, using tetrabutylammonium iodide as the supporting electrolyte, the substances being dissolved in methanol.

Table 7 Half wave potentials

Compound	$E_{\frac{1}{2}}$ (V) (= first prominent wave inflection)
Acetic acid	-0.076
Sodium acetate	-0.024
Ethyl acetate	0.31
Acetamide	-0.137

In view of the ambiguity of interpretation of the polarographic results (many inflections were observed for each compound), these figures are not used in the following discussion.

Table 8 and fig. 6 show the correlation obtained between the quenching factor and the EA of the carbonyl group for the indoleacetic series.

These results strongly support a theory involving electron donation by the excited indole to the side chain carbonyl group.

Table 8

Compound	Side chain EA	Q	$\frac{Q(\text{skatole})}{Q(\text{compound})}$
Skatole	-	0.325	(1.00)
Indoleacetic acid	14.58	0.13	2.5
Indoleacetate anion	0	0.23	1.39
Ethyl indoleacetate	17.24	0.12	2.65
Indoleacetamide	0	0.23	1.37

Table 9

Compound	τ(ns)	Q/τ
Indole	4.3	0.13
Ethyl indoleacetate	0.7	0.17

General Discussion.

These results may now be considered in conjunction with the theories mentioned at the beginning of the chapter. The electron scavenger theory of quenching of Steiner and Kirby[38] is in agreement with the present results in the sense that formation of a DA complex by intramolecular charge transfer is equivalent to removal of an electron by a scavenger which is not then held at close range to the molecule.

The formation of a DA complex does not in itself explain the mechanism of quenching of fluorescence. The quantum yield is given by eq. 4 :

$$Q = \frac{k_F}{k_F + k_{NR}} \qquad (4)$$

where k_F is the radiative rate constant and k_{NR} represents a sum of nonradiative constants k_{IC}, k_{ISC}, k_Q, respectively internal conversion, intersystem crossing and quasichemical quenching (e.g. photoreaction) constants.

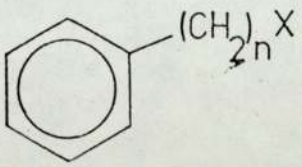
That quenching is not because of a change in the value of k_F is indicated by the constant values of Q/τ ($=k_F$), where τ is the measured lifetime. This was noted by Weinryb and Steiner for a series of tryptophan derivatives[130], and values are shown in table 9 for some compounds studied here.

Cowgill's hypothesis[8] of enhanced vibrational coupling between S_0 and S_1 is equivalent to the postulation of an increase in k_{IC}. He suggested this as an alternative to an increase in k_{ISC}, since Weinryb and Steiner[130] had shown that the fluorescence/phosphorescence ratios for a series of

tryptophan derivatives (of varying fluorescence yields) was constant. However, their fluorescence/phosphorescence ratios were measured at liquid nitrogen temperatures, and if quenching involves initial physical contact of the side chain with the nucleus, this movement would be inhibited at low temperatures. The absence of quenching at low temperatures[130] is not contrary to the present hypothesis, but invalidates Cowgill's assumption that intersystem crossing is not enhanced.

Internal conversion is thought to be of low significance in polycyclic aromatic hydrocarbons (but of greater significance in benzene and its derivatives)[131]. It may become important when the potential energy diagrams for the ground and excited states approach or cross each other (ref. 7, p. 297). A mechanism of this type was suggested by Eisinger and Navon[33] for the quenching of tryptophan in water. However it is noticeable that the spectra of all the 3 - substituted indoles are very similar, varying only in intensity. This would imply similar excited state energies, and large variations in k_{IC} are therefore unlikely.

Fluorescence quenching in a related series of compounds:



has been investigated by Tournon and El - Bayoumi[132]. These authors interpreted quenching in terms of intramolecular charge transfer between the aromatic ring and the substituent.

As in the case of the indoles, a steric requirement was found, i.e. the quencher X must be capable of approaching the ring if quenching was to occur. They also claimed that intramolecular charge transfer in these molecules leads to an increase in intersystem crossing, an increase in the phosphorescence rate constant, and an increase in the phosphorescence/fluorescence ratio. Their fluorescence and phosphorescence measurements supported these claims.

It has been recognised for some time that intersystem crossing is enhanced in charge transfer complexes which are stable in the ground state[133]. These results obtained here indicate that this conclusion can be extended to complexes which are formed in the excited state.

An increase in k_{ISC} would increase the triplet yield, and it was attempted to measure the triplet yields of some 3 - substituted indoles, using the technique of Lamola and Hammond involving the triplet sensitised photoisomerisation of piperylene[134]. This technique is described in the experimental section. Values obtained were not reproducible, and further work is required before triplet yields of indoles can be quoted.

Results indicate that solvation by a polar solvent is a requirement of quenching. As stated at the beginning of this chapter, Cowgill's results[3] indicated that the carbonyl group must be solvated. This was based on studies involving the addition of organic solvent to a water solution of an indole derivative. Water, however, behaves anomalously when compared with alcohols, possibly because of the ready formation of

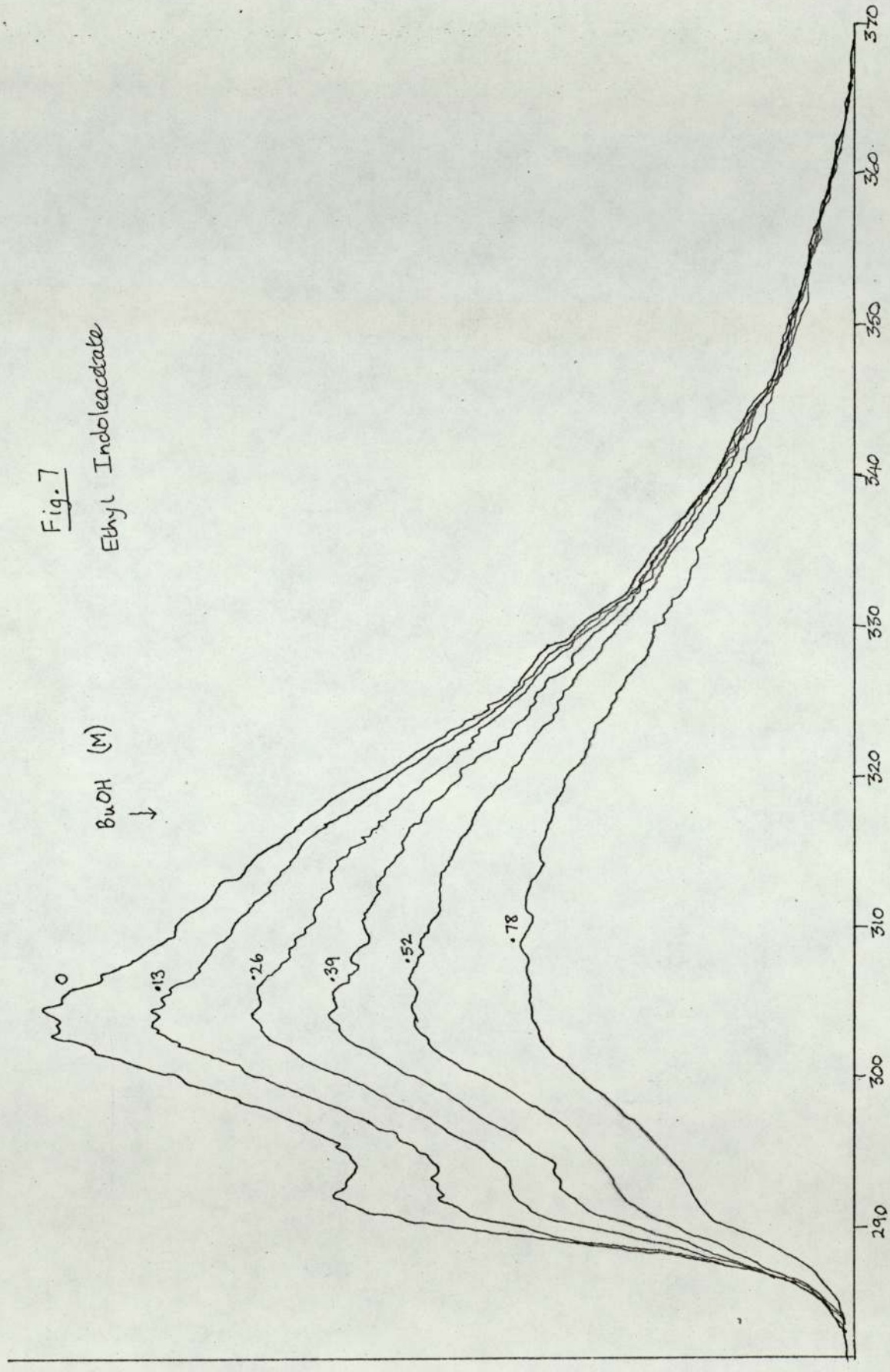
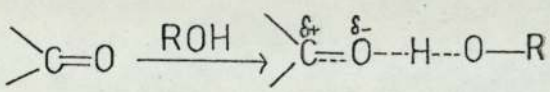


Fig. 7
Ethyl Indoleacetate
BuOH (M)
0
.13
.26
.39
.52
.78
Rel. F.
290
300
310
320
330
340
350
360
370

oligomers of water. Fig. 7 presents the spectra of ethyl indoleacetate, obtained on titration with 1 - butanol in cyclohexane solution, as was done in the exciplex titrations mentioned earlier. As can be seen from fig 7, only a small concentration of butanol is needed to quench the fluorescence of this compound. This correlates with the formation of the exciplex. To conclude that carbonyl solvation is not required, but only indole exciplex formation, requires proof that carbonyl hydrogen bonding does not occur at such low butanol concentrations. In principle, I.R. measurements of the carbonyl frequency could be used, A shift of 9K was noted by Searles et. al. on hydrogen bonding of ethyl acetate to methanol in CCl_4[135]. In practice, absorption by the solvent precludes measurements at such low concentrations. However, it may be pointed out that Searles et. al. obtained a shift of 9K for approximately molar solutions of ethyl acetate and methanol in CCl_4. 9K represents a relatively weak interaction and it is likely that on dilution, at room temperature, the bonding would become less significant. This may be compared with studies on hydrogen bonding in alcohols[123], when much larger shifts may be obtained, and yet intermolecular bonding virtually disappears below 0.02M at room temperature. It therefore seems likely that solvation of the carbonyl group is not the principal reason for the requirement of a polar solvent.

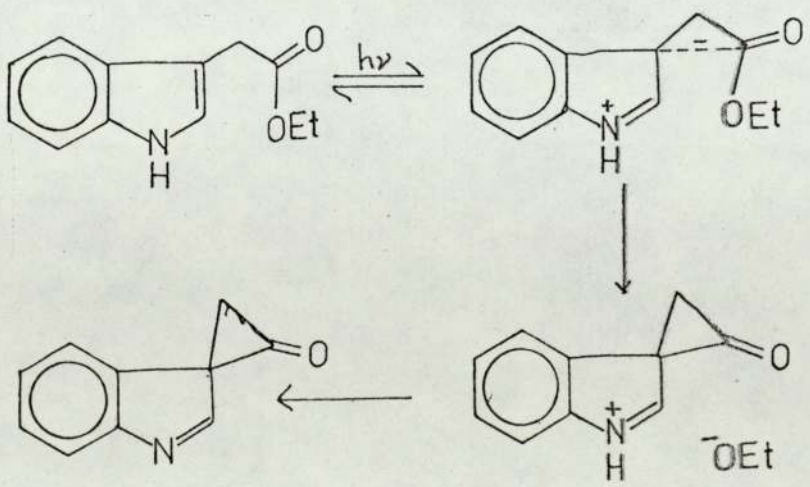
Nevertheless, solvation of the carbonyl group will occur at higher hydroxylic solvent concentrations. Such solvation causes

a shift to lower frequencies of the C=O absorption at ca. 1700K[135]. A shift in this direction has been correlated (for a series of ketones) with an increase in carbonyl bond order[136]. This would be expected, as hydrogen bonding will increase the contribution of ionic structures to the resonance hybrid.



This should aid quenching, since the increase in positive charge on the carbonyl carbon will increase its electron affinity.

It is likely that the site of charge transfer is localised on the indole molecule. Similar arguments may be applied here as were applied in the discussion on the exciplex. Thus, quenching in ethyl indoleacetate could perhaps lead to a photochemical reaction (increase in k_Q mentioned earlier) of the type:



Again, a preliminary photolysis of ethyl indoleacetate failed to produce an indolenine chromophore. The investigation was not pursued further.

Conclusions.

The indole molecule on excitation fluoresces in a non - polar solvent with a quantum yield of ca. 0.55. On addition of a hydroxylic solvent, the excited indole molecule can react with a solvent molecule producing an exciplex. This has a tendency to lose electrons (but only a weak tendency in the absence of further environmental effects[137]), causing quenching of the fluorescence. In the presence of a carbonyl group which is sterically capable of acting as an intramolecular electron acceptor, the exciplex can form a donor - acceptor type of complex, which is (presumably) non - fluorescent. (Any fluorescence from this, a new species, would be expected to be at a different wavelength). Formation of this complex facilitates energy dissipation, most likely by intersystem crossing to the triplet state, although no absolute verification of this has been obtained.

TECHNIQUES AND EXPERIMENTAL DETAIL

A. THE DETERMINATION OF THE QUANTUM YIELD OF FLUORESCENCE.

The quantum yield of fluorescence has been defined[138] as the fraction of molecules that emit a photon after direct excitation by the source. On the assumption that excitation of a molecule is a single photon process, (and using a 150W or a 250W xenon arc lamp of relatively low intensity after monochromation, the probability of multiphotonic absorption occurring is low) the quantum yield becomes the ratio of the number of emitted to absorbed photons. Thus, measurements of the fluorescence intensity and absorption are required.

The measurement of photoluminescence quantum yields has been discussed in detail by Demas and Crosby[138]. Absolute methods were of no use in this work because of the difficulty of obtaining high accuracy, and the need only for relative values. A method of comparison against a standard compound of known quantum yield, was required. As Demas and Crosby pointed out, there are two general techniques possible, one using optically dense measurements, the other optically dilute measurements. The errors introduced in optically dense measurements are high - concentration quenching, solute aggregation, reabsorption - reemission phenomena etc. Optically dilute measurements, with the absorbance less than 0.02, are much less prone to these errors. In this case, the fluorescence intensity F (quanta per unit time emitted in all directions) is given by:

$$F = I_o(1 - 10^{-El}).Q \qquad (1)$$

where I_o = incident intensity in quanta per unit time;

E = molar extinction coefficient × concentration (εC);

l = optical depth;

Q = quantum yield.

Expanding the exponential of (1) in a power series, and neglecting higher order terms in El since these are small,

$$F = I_o . 2{\cdot}303El.Q \qquad (2)$$

This was derived by Parker and Rees[139], who went on to show that, comparing two compounds,

$$\frac{F_2}{F_1} = \frac{Q_2}{Q_1} \times \frac{A_2}{A_1} \qquad (3)$$

where A = absorbance = El, and the subscripts denote compounds 1 and 2.

The use of a standard Aminco - Bowman spectrophotofluorimeter, however, is not straightforward with respect to the application of this formula. The rest of this description refers to the use of this instrument for quantum yield determination. It is accepted that this is not necessarily the best instrument for the determination of quantum yields; nevertheless, the ubiquity of the machine warrants the design of a method for obtaining quantum yields of as high accuracy as possible.

The use of eq. (3) requires dilute solutions to avoid the error caused by the approximation made. As Demas and Crosby point out[138], however, provided that $A_1 - A_2$ is $\pm 0{\cdot}02$ A or less, the error in A_2/A_1 is $\leqslant 2\%$. Since it is impossible

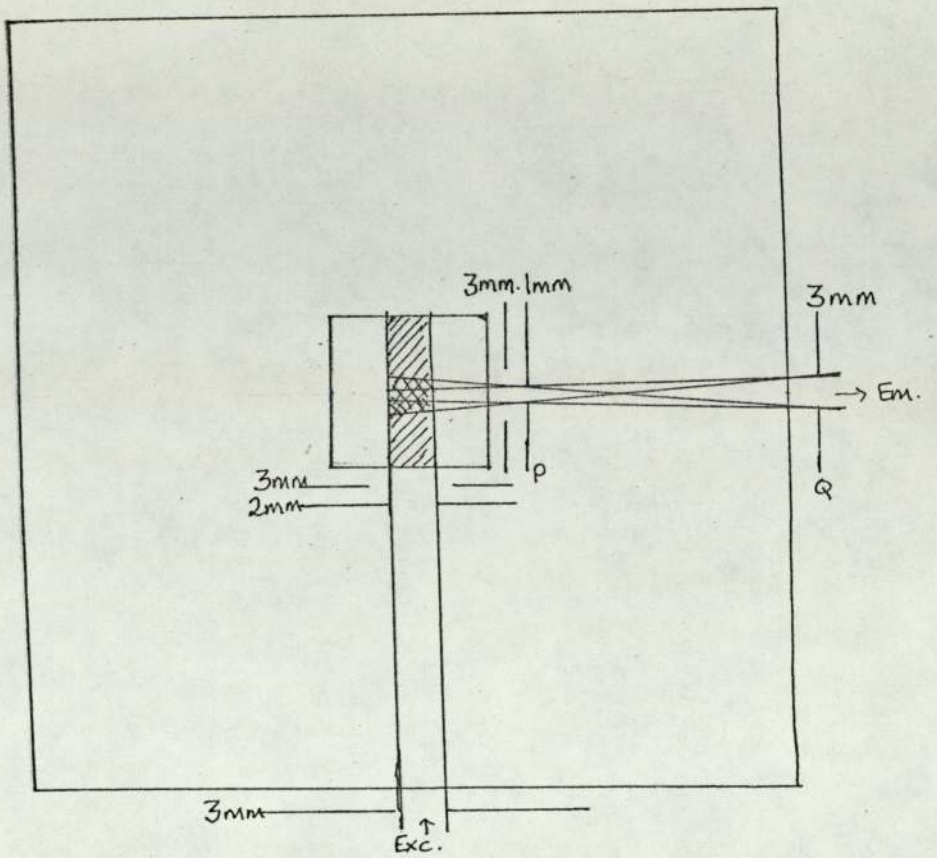
to measure very low absorbances with high accuracy, it is necessary to use solutions of optical density greater than 0·2. Solutions used in this work were in general nitrogenated, a process which reduces the solvent absorption by more than 0·01 per cm at 250nm, with reference to an air equilibrated sample.

It was considered that the Beckman Acta V used here gave absorbances to $\pm$ 0·005 or better. Thus 2% accuracy was achieved by using solutions of absorbance greater than or equal to 0·25, and, to minimise concentration effects, absorbances were kept as low as possible above this value.

Use of solutions of this magnitude of density was found to give rise to some problems of self absorption. The procedure of nitrogenation of solutions, although performed with N_2 previously passed through a bottle of solvent, did cause some alteration in the volume of solvent. Hence it was difficult to prepare solutions of absorbance 0·25 $\pm$ 0·01; a method accommodating a wider variation in absorbance was required. It was therefore necessary to use eq. (1).

In eq. (1), F represents the total emitted intensity. The measured intensity is a fraction of this, which, with the geometry shown in fig. 1, is seen to vary depending on the solution absorbance. Thus, for low absorbances, emission will be constant along the excitation path, but for high absorbances, emission will be greater nearer the light entrance to the cell.

Fig 1



Approximating the geometry to a two dimensional system, the configuration of the Aminco SPF can be represented as in fig. 2, which is an enlargement of the optical path shown in fig. 1, drawn on Cartesian axes.

The fluoresced light is collected from regions A, B and C, with an angular distribution between the boundary lines:

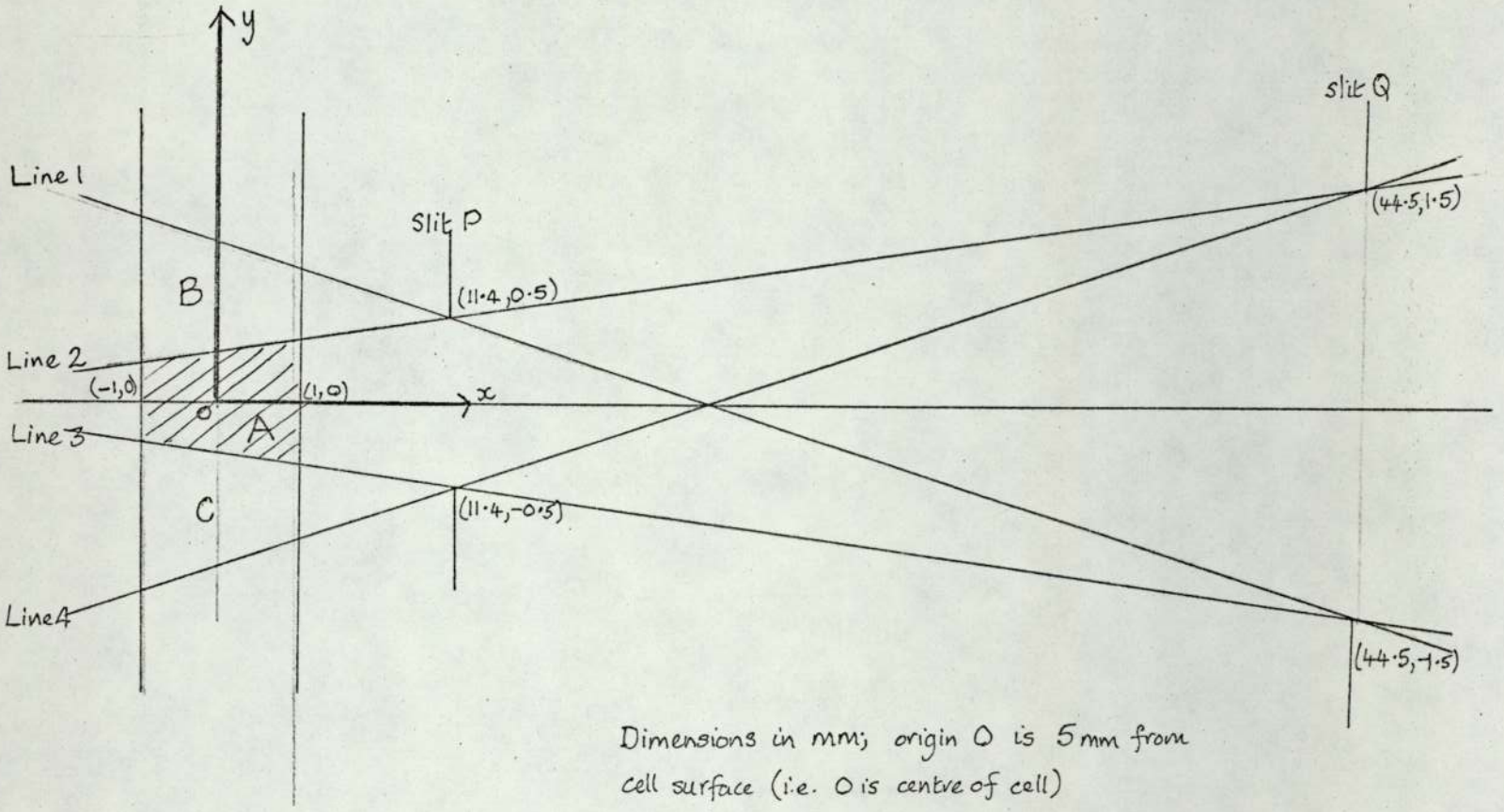
Line 1 $$y = \frac{39.35 - 2x}{33.1} \quad (4)$$

Line 2 $$y = \frac{x + 5.15}{33.1} \quad (5)$$

Line 3 $$y = \frac{-(x + 5.15)}{33.1} \quad (6)$$

Line 4 $$y = \frac{2x - 39.35}{33.1} \quad (7)$$

FIG. 2



Dimensions in mm; origin O is 5 mm from cell surface (i.e. O is centre of cell)

The excitation beam is approximately bounded by the lines

$$x = \pm 1{\cdot}0 \qquad (8)$$

Consider emission from a point (x,y) within regions A,B,C. Then the total fluoresced intensity from a unit of volume centred on point (x,y) is given by

$$dF_{tot} = I'_o . 2{\cdot}303 . E . dy . Q \qquad (9)$$

but

$$I'_o = I_o . 10^{-E(y/10 + 0{\cdot}5)}$$

$$= I_o \exp(-0{\cdot}2303Ey) . \exp(-1{\cdot}1515E) \qquad (10)$$

The measured fluorescence intensity from this unit of volume is

$$dF_{meas} = \frac{\theta(x,y)}{2} . I_o \exp(-0{\cdot}2303Ey) . \exp(-1{\cdot}1515E) . 2{\cdot}303E . dy . Q \qquad (11)$$

where θ is the angle subtended by (x,y) at the slit system, i.e. is the angle between lines joining:

(x,y) to (44·5, ±1·5) (region A)

(x,y) to (11·4,0·5) and (44·5,-1·5) (region B)

(x,y) to (11·4,-0·5) and (44·5,1·5) (region C)

The total measured fluorescence intensity is obtained by integrating eq. (11) throughout the regions A,B and C.

$$F_{meas} = \exp(-1{\cdot}1515E) . 2{\cdot}303E . Q . I_o . \int_{-1}^{1}\int_{X}^{Z} \exp(-0{\cdot}2303Ey) \frac{\theta(x,y)}{2} dy dx \qquad (12)$$

For two substances, then, eq. (3) becomes (cancelling out constants $2{\cdot}303 . E . I_o$)

$$\frac{(CF)_2 \cdot F_2}{(CF)_1 \cdot F_1} = \frac{Q_2}{Q_1} \cdot \frac{A_2}{A_1} \qquad (13)$$

where the 'correction factors' CF (representing the factors

required to correct the emitted intensity for self absorption and geometrical effects) are given from eq. (12) by

$$CF = \exp(-1{\cdot}1515E).\int_{-1}^{1}\int_{X}^{Z} \frac{\theta(x,y)}{2}.\exp(-0{\cdot}2303Ey).dy.dx \quad (14)$$

where X and Z are functions of x, depending on the region.

Angular function $\theta(x,y)$

Region A. By conventional coordinate geometry,

$$\tan\theta_A \simeq \theta_A \simeq \frac{(y-1{\cdot}5)(x-44{\cdot}5) - (y+1{\cdot}5)(x-44{\cdot}5)}{(x-44{\cdot}5)(x-44{\cdot}5) + (y-1{\cdot}5)(y+1{\cdot}5)}$$

$$\simeq \frac{-3{\cdot}0(x-44{\cdot}5)}{(x-44{\cdot}5)^2 + y^2 - (1{\cdot}5)^2} \quad (15)$$

but $|x| \leqslant 1{\cdot}0$ and $|y| \leqslant 1{\cdot}0$

therefore $\theta_A \simeq \frac{132}{1934}$

$$\underline{\theta_A \simeq 0{\cdot}068 \text{ rad.}}$$

similarly for Region B.

$$\theta_B = \frac{39{\cdot}35 - 2x - 33{\cdot}1y}{x^2 + y^2 - 55{\cdot}9x + y + 506{\cdot}55}$$

but $|x| \leqslant 1{\cdot}0$ and $|y| \leqslant 1{\cdot}25$

therefore $$\underline{\theta_B \simeq \frac{39{\cdot}35 - 2x - 33{\cdot}1y}{-55{\cdot}9x + 506{\cdot}55}} \quad (16)$$

Region C similarly

$$\underline{\theta_C \simeq \frac{39{\cdot}35 - 2x + 33{\cdot}1y}{506{\cdot}55 - 55{\cdot}9x}} \quad (17)$$

Correction factors

Region A

$$CF_A = \exp(-1{\cdot}1515E).\int_{-1}^{1}\int_{X}^{Z} \frac{0{\cdot}068}{2} \exp(-0{\cdot}2303Ey).dy.dx \quad (18)$$

where $Z = \frac{x + 5{\cdot}15}{33{\cdot}1}$, and $X = \frac{-(x + 5{\cdot}15)}{33{\cdot}1}$

$$\therefore\ CF_A = \frac{27{\cdot}02}{E^2}.\sinh(0{\cdot}00696E).\sinh(0{\cdot}03587E).\exp(-1{\cdot}1515E) \qquad (19)$$

Regions B and C

$$CF_B = \frac{\exp(-1{\cdot}1515E)}{2}.\int_{-1}^{1}\int_{X'}^{Z'} \frac{(39{\cdot}35-2x-33{\cdot}1y)}{(506{\cdot}55 - 55{\cdot}9x)}.\exp(-0{\cdot}2303Ey).dy.dx \qquad (20)$$

where $Z' = \frac{39{\cdot}35 - 2x}{33{\cdot}1}$ and $X' = \frac{x + 5{\cdot}15}{33{\cdot}1}$.

$$CF_C = \frac{\exp(-1{\cdot}1515E)}{2}.\int_{-1}^{1}\int_{X''}^{Z''} \frac{(39{\cdot}35-2x+33{\cdot}1y)}{(506{\cdot}55 - 55{\cdot}9x)}.\exp(-0{\cdot}2303Ey).dy.dx \qquad (21)$$

where $Z'' = \frac{2x - 39{\cdot}35}{33{\cdot}1}$ and $X'' = -\frac{(x + 5{\cdot}15)}{33{\cdot}1}$

Eqs. (20) and (21) cannot easily be calculated analytically. Consequently, a computer program was written to evaluate numerically these integrals, together with eq. (19).(see the appendix). The program output the contributions of CF_A, CF_B and CF_C to the total correction factor CF, for absorbances in steps of 0·05 from 0·05 to 1·45. It showed, in fact, that for absorbances up to 1·45, the approximate correction factor $\exp(-1{\cdot}1515E)$ (i.e. $10^{-(A/2)}$) is in error only by 1·4% with respect to the total CF.

Thus, it was necessary to correct for self absorption up to the centre of the cuvette, but correction for variation in intensity along the element of cell viewed by the slit system was found to be negligible.

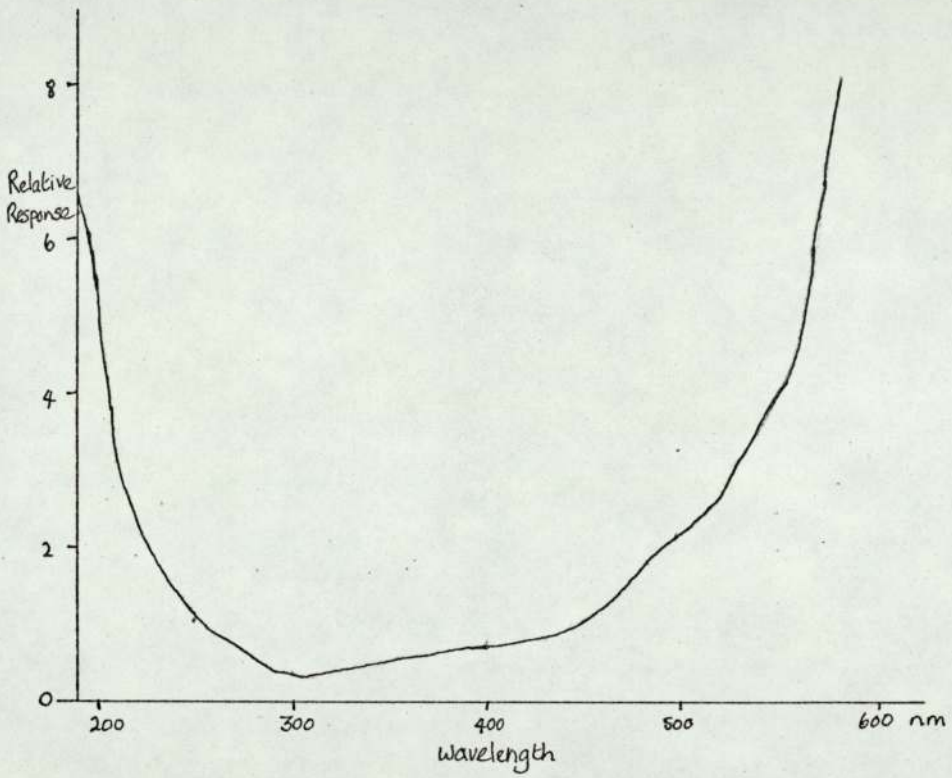
The front face optical system was recently considered with respect to the angular distribution of emitted light, by Mode and Sisson[140], who listed correction factors for the front face geometry.

The above discussion is concerned only with the accurate measurement of absorbance or fluorescence intensity. Further adjustments were needed to correct for the variation in the optical response with wavelength. Thus the absorbed light was not precisely monochromatic; a 2mm slit used gave a bandwidth of several nanometres. The same excitation settings were used throughout a set of readings; the slits were as in fig. 1, with the wavelength set at 265nm for cyclohexane solutions and 275nm for other solutions. In both cases, the excitation band envelope was obtained by scattering the light with glycogen solution[138] into the emission monochromator set with slits 3·0, 0·5, 3·0 and PM 0·5 mm (in the order of the light path). The bandwidth of the emission monochromator was found using a low pressure mercury lamp on the 363nm line, assumed to have negligible width. The observed excitation function was deconvoluted against this emission band envelope (see appendix for the computer program DECONV), to give the true excitation envelope. This was then convoluted with the absorption spectrum (obtained on the Beckman Acta V) to give values of $(A_{\lambda_{max,obs}} / A_{\text{applicable to fluorimeter}})$, from which measurements of maximal absorption on the Acta V could be converted to actual absorption figures for each compound. (Program CONVOLUTE is appended).

The fluorescence spectrum was corrected for variations in emission monochromator/photomultiplier combination response with wavelength, and the area under the spectrum was evaluated

trapezoidally. The correction factors were established by (a) using a rhodamine B quantum counter[138] ($3g.l^{-1}$ in ethane - 1,2 - diol) to determine the output of the xenon lamp, and (b) scattering the light using glycogen solution (or direct reflection from a ground glass surface was found to give similar results) into the emission monochromator.

Fig. 3 Emission monochromator/PM spectral response



These factors were fed with the observed intensity readings at 1, 2 or 4nm intervals into a computer program (FLUCORR, appended), which was written to give corrected spectra in terms of photon irradiance ($h\bar{\nu}.s^{-1}.m^{-2}$) and also spectral photon irradiance ($h\bar{\nu}.s^{-1}.m^{-2}.nm^{-1}$ or $h\bar{\nu}.s^{-1}.m^{-2}.K^{-1}$). The

integrated intensities were evaluated in similar terms. Results were calculated using the integral of photon spectral irradiance with respect to wavelength.

These units and the corrections have been discussed by Melhuish[141].

The standard refractive index correction[138] was applied where necessary, but correction was not made for polarisation effects. These were considered to be negligible, since all solutions measured were of small molecules in mobile solvents, and the spectra were similar in wavelength range.

Wavelength calibration of the emission monochromator was performed using a low pressure mercury 'Pen - Ray' lamp, which was placed behind the exit slit of the excitation monochromator, with narrow slits in all positions; glycogen solution was used to scatter the mercury light into the emission monochromator, which was thus calibrated under the correct geometrical conditions. The excitation monochromator was then calibrated using the xenon arc lamp, with glycogen scattering.

The Aminco - Bowman fluorimeter was modified as follows:

i) The PM tube was a selected EMI 6781B, instead of the less sensitive RCA 1P28;

ii) The emission grating was blazed at 300nm (as was the excitation grating).

iii) The wavelength drive battery was replaced by a zener diode regulated 5·65v power supply designed by the author;

the circuit diagram is appended.

iv) A Hewlett - Packard 7035B XY plotter was used to record the spectra.

B. THE DETERMINATION OF THE TRIPLET QUANTUM YIELD.

The triplet quantum yield (triplet yield, Q_T) may be defined as the fraction of molecules that cross over to the triplet state after direct excitation to the excited singlet state by the source. This can be expressed in terms of the rate constants[93, chap 6] as

$$Q_T = \frac{k_{ISC}}{k_{ISC} + k_F + k_{NR}} \qquad (1)$$

where k_{ISC} is the intersystem crossing rate constant, and the denominator is the sum of rate constants for all the deactivation processes from the excited singlet state.

A determination of the triplet yield, then, involves measurement of the amount of light absorbed by the sample, and the concentration of triplets produced. It is the latter measurement which poses the greater problem.

Perhaps the most direct method to determine the triplet concentration is to measure the triplet - triplet absorption spectrum (i.e. excitation from the T_0 state, initially populated from the excited singlet state, to T_n, $n > 0$). This requires initial knowledge of the $T_0 - T_n$ extinction coefficient, which may be difficult to determine accurately. Methods based on this have been reviewed by Birks[93, chap 6], and all of these involve at some stage a flash photolysis experiment.

Other methods involve some other species which interacts with the triplets produced. These methods involve the assumption that the interaction depends only on the multiplicity of the molecule, and not on its identity, i.e. triplets of different

compounds will interact with equal efficiency. Some of these methods have also been described by Birks[93].

The method chosen here, because of its applicability to apparatus already available, was that of Lamola and Hammond[134]. This method utilises the isomerisation of penta - 1,3 - diene (piperylene), which is a triplet sensitised reaction[149,150,151]. Thus, if a solution containing the absorbing species whose triplet yield is required, together with pure cis - piperylene is irradiated, then the triplet yield will be proportional to the initial rate of isomerisation of the cis - piperylene to its trans isomer.[151] The proportionality constant may be obtained by comparison with a substance (benzophenone) of known triplet yield.

The following experimental procedure was adopted.

Piperylenes dissolved in cyclohexane were estimated by GLC using 2 - methylbut - 2 - ene as internal standard. (An unidentified impurity was present in the cyclohexane at low concentrations; this impurity peak was used as an alternative to the methylbutene with one batch of cyclohexane). A 15ft x 1/8" 15% dimethylsulpholane metal column was used in a Perkin Elmer F11 chromatograph, temperature 45^{o}, nitrogen pressure 25 $lb.ft^{2}$. A sample chromatogram obtained under these conditions is shown in fig. 1.

Experimental samples were made up as follows. 0.56ml of 0.02M sensitiser (benzophenone, naphthalene or the indole under test) + 0.2ml of 0.1M cis - piperylene (both compounds in either methanol or cyclohexane) were placed in a cylindrical

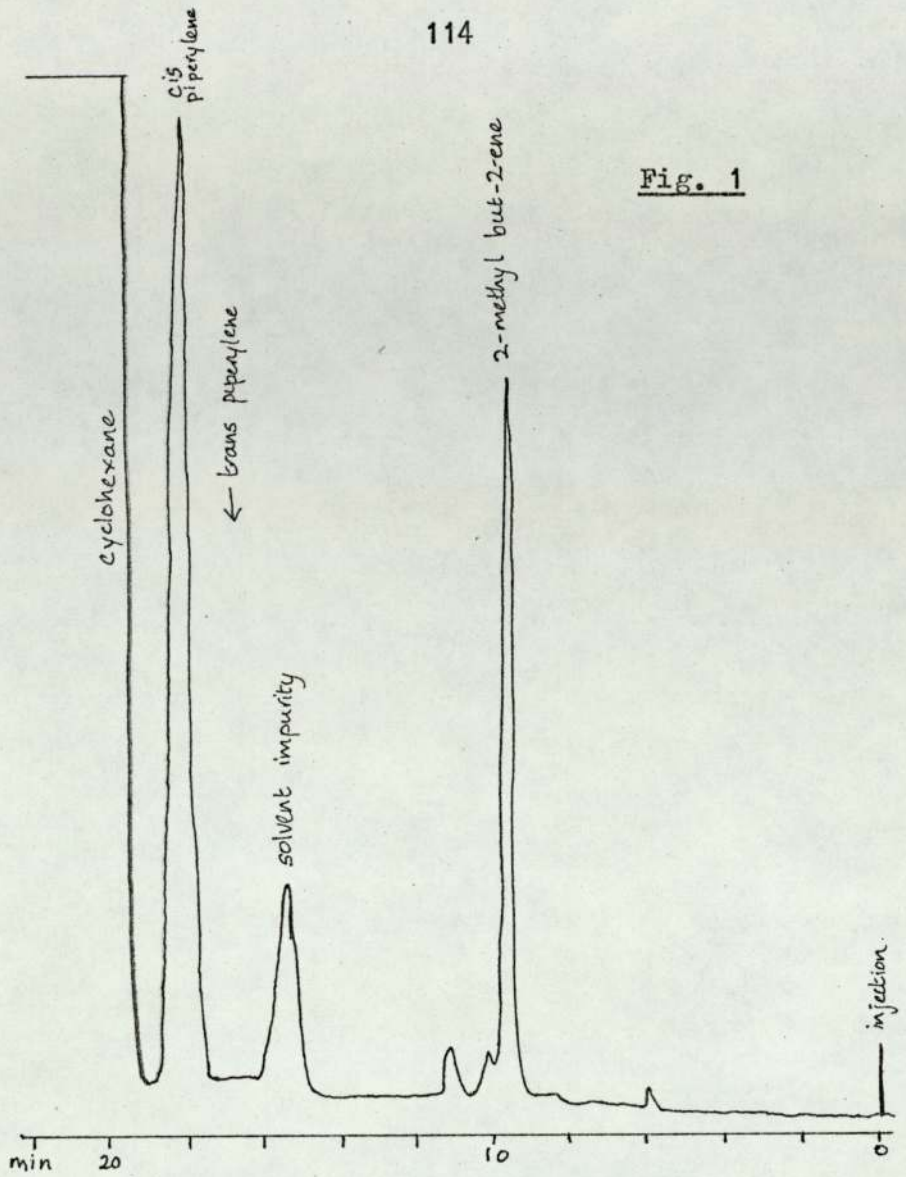
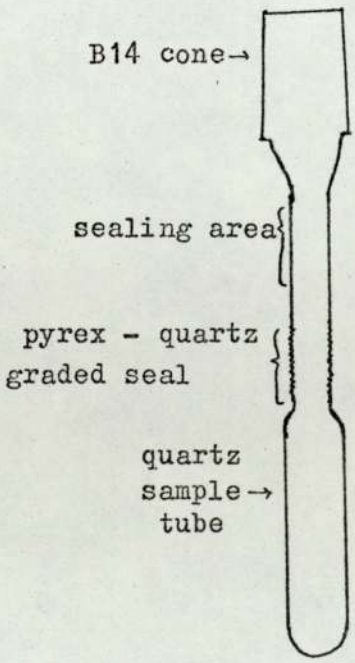

cyclohexane
cis piperylene
← trans piperylene
solvent impurity
2-methyl but-2-ene
injection
min
20
10
0

Fig. 1

Fig. 2


B14 cone→
sealing area
pyrex - quartz
graded seal
quartz
sample→
tube

quartz sample tube (fig. 2) which was then deoxygenated by several freeze - pump - thaw cycles to 10^{-3}mm Hg, and sealed under vacuum. The samples (2 of each compound) were irradiated in a photochemical 'merry - go - round' apparatus (Applied Photophysics Ltd.) for a period determined from initial experiments with benzophenone and naphthalene. (This was found to be ca. 1h for the lamp and filter arrangement used, and was sufficiently long to give an isomerisation of about 5 - 10%). The lamp, a 250W Hg arc lamp, was filtered by means of a Locarte LF1 bandpass (255 - 370nm) filter in series with 2cm of a solution of Ni^{2+} in ammonia (7g $NiCl_2.6H_2O$ made up to 25ml with water; this solution was treated with 0.880 ammonia until clear, the clear violet solution acting as the filter).(fig. 3)

Samples of each compound were withdrawn at the period mentioned above; their duplicates were irradiated for a further equal period. All samples were stored in the dark at $0^{\circ}C$ until estimation. Cyclohexane samples were analysed by direct injection into the GLC; methanol samples were first extracted into 2ml cyclohexane, which was washed twice with water to remove any methanol, then dried with $MgSO_4$ before injection.

Standard ferrioxalate actinometry[152] showed that only a negligible amount of light passed through the samples of benzophenone or naphthalene, so each compound absorbed the same amount of light.

The initial rate of isomerisation was evaluated from the two points obtained from the GLC analysis. These rates gave triplet yields as in table 1, using Q_T(benzophenone) = 1.00 as standard.

FIG. 3 FILTER SPECTRA

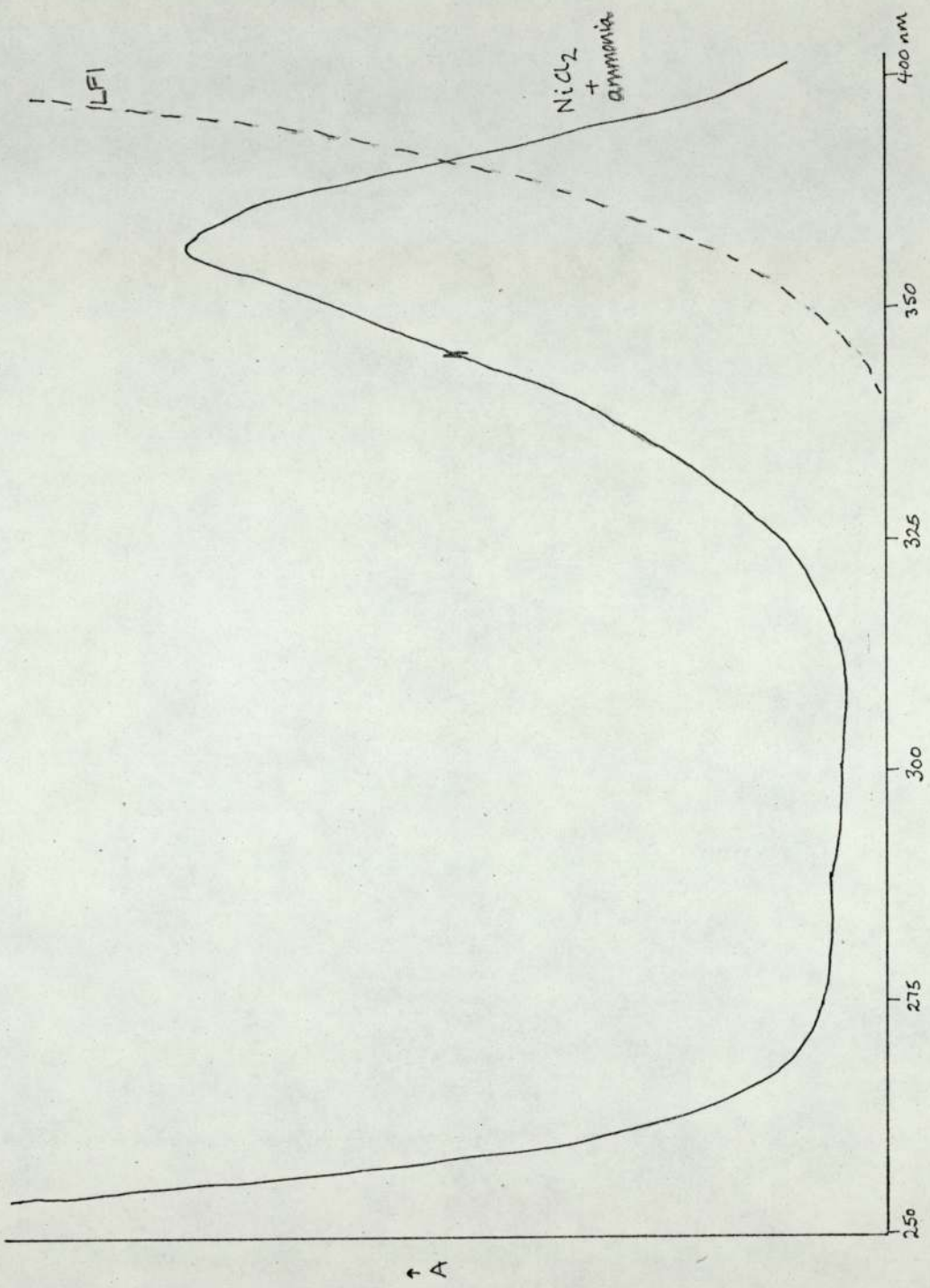


Table 1 Triplet yields (cyclohexane)

Benzophenone	(1.00)
Naphthalene	0.6 (lit. 0.80/ethanol, 0.4,0.82/benzene[93])
Skatole	0.2

The results for skatole were not repeatable as were those of naphthalene. However, skatole transmits at wavelengths above 310nm in these concentrations, and a better filtering system was therefore needed. A suitable filter was not found, and an experiment using a monochromator was unsuccessful, probably because of the low output of the monochromator as compared with a filter.

It is suggested that there is scope for improvement of this technique in this application, and further work on these lines may give triplet yields of considerable value in the understanding of the photophysics of indoles.

C. THE DETERMINATION OF FLUORESCENCE LIFETIMES.

Consider a solution of some absorbing compound, which is being irradiated so that molecules in solution are excited to an upper singlet state. Then on the instantaneous termination of irradiation at time t = 0, the system will decay (by a combination of radiative and non - radiative processes), and the concentration of excited species A^* will be given by:

$$[A^*] = [A^*]_0 \cdot \exp -(k_R + k_{NR})t \qquad (1)$$

where $[A^*]_0$ is the concentration of A^* at time t = 0.

The lifetime is defined by

$$\tau = 1/(k_R + k_{NR})$$

for the above process. If the system contains more than one emitting species, then the decay as measured by the fluorescence intensity I ($I \propto [A^*]$) will be a sum of exponentials instead of a single component, with one exponential for each emitting species.

Consequently, a method for the determination of fluorescence lifetimes should be capable of giving all the values of τ and their relative amplitudes.

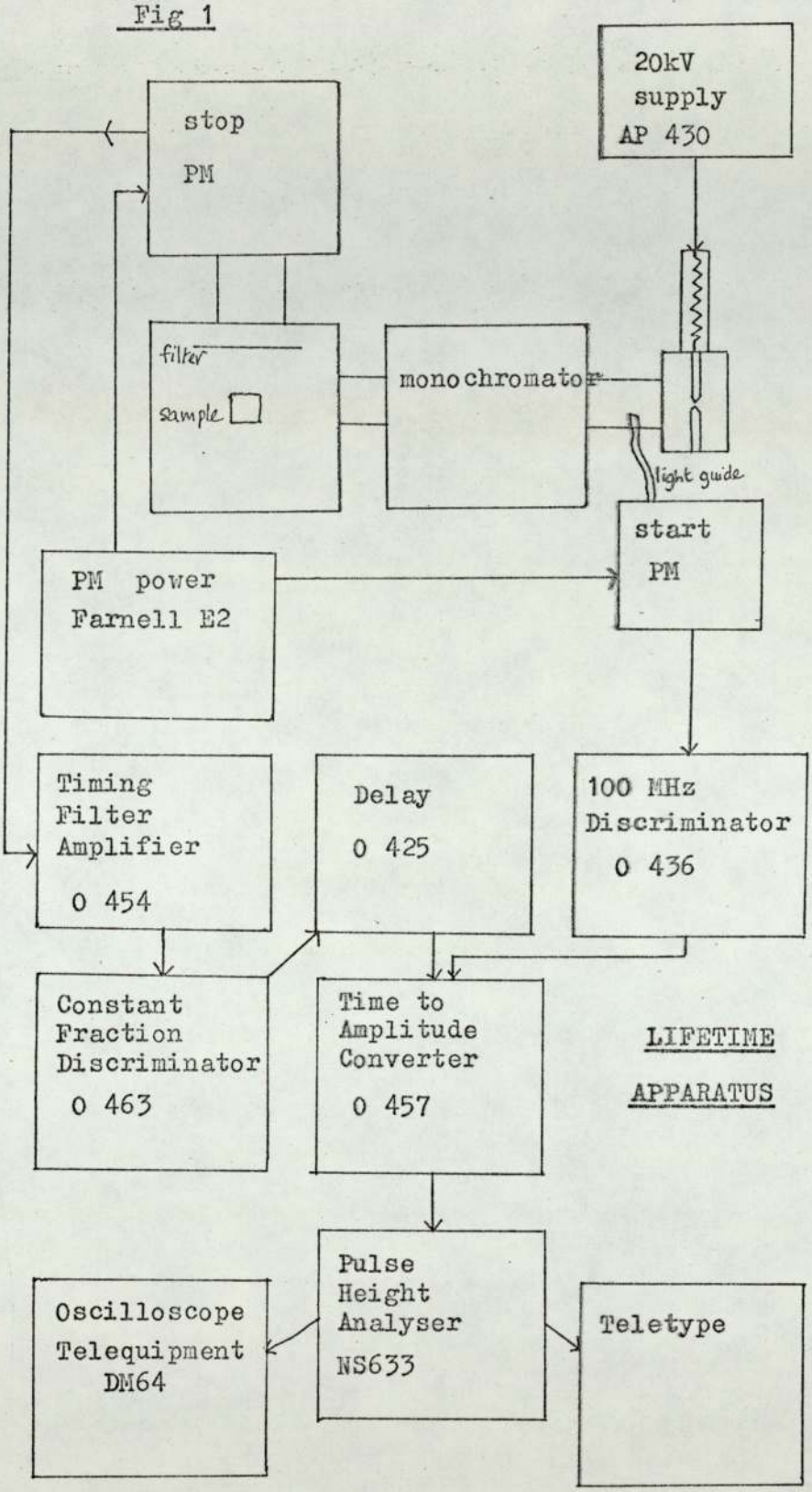
Methods have been reviewed in some detail by Ware[142]. They fall into two classes, namely pulse methods and phase shift methods. Pulse methods rely on observing the decay of the system when the excitation source is switched off (ideally) instantaneously. In principle, therefore, these are direct methods, and will give a decay curve which can be analysed directly into its lifetime components, limited only by the

noise on the curve. In practice, however, termination of excitation takes a finite length of time, and the lifetimes are short enough to be comparable to this time. Also, one is working near the limits of electronic instrumental resolution on a time scale. Thus, the observed curve is distorted by the instrumental response function, which must be corrected for. The phase shift method, on the other hand, uses an excitation beam which is modulated sinusoidally, a procedure which can be done with considerable accuracy in waveshape. However, analysis of the data requires prior knowledge of the functional form of the decay (i.e. the number of components), and there are also other problems of instrumentation which are discussed by Ware[142].

The method used in this work was a pulse method, using the single photon counting technique with a nanosecond flash lamp. Since the method has been described by Ware[142] and also Lewis et. al.[143], little detail will be given here other than a description of our apparatus and some comments on the method of operation not covered in the above references.

Fig. 1 gives a block diagram of the apparatus. The spark lamp (Applied Photophysics Ltd.) was operated with hydrogen at 5atm., and an EHT of 20kV. Light from the lamp was sampled via a small mirror and fibre optic guide with a R.C.A. 1P28 photomultiplier (PM). The light was monochromated (an f/4 200mm focal length symmetrical Czerny - Turner unit with 300nm blazing on the grating) and focussed on to the sample in a 1cm cell or a sealed (stoppered) cylindrical tube. Emission at

Fig 1



AP = Applied Photophysics Ltd.
O = Ortec

right angles was filtered (to remove scattered light) and focussed onto the photocathode of a R.C.A. 8850 fast PM, operated at 2.5kV.

The sampled start pulses were found to be several volts in amplitude, so the 100 MHz discriminator setting could be as high as 2V to cut out low voltage noise pulses. The timing filter amplifier was set to give a gain of about 1.5 on the stop pulses which were only about 1V in amplitude.

Some comment on the method of setting the discriminator level on the stop pulse line is necessary. With a sample in position, and the instrument operational, the rate of counting may be varied by either altering the optical settings (i.e. slit widths etc.), or by altering the stop discriminator level. With this particular stop PM at room temperature, a high noise level was recorded with the light path blanked off. Furthermore, setting the discriminator level high enough to cut out most of the noise was found to cut out the signal also. Consequently, the initial procedure adopted was to adjust the discriminator level to an optimum value where blanking off of the light beam caused the greatest change in count rate. This was quite a high setting, and further consideration of this indicated that it was a potential source of error. Standard practice[142] is to adjust the count rate so that approximately one count is recorded per 20 start pulses. This means that the chance of two photons impinging on the stop PM photocathode simultaneously (i.e. within the response time of the detector system) is very low. Nevertheless, some of this 'pulse pile -

up' will occur. This may give rise to relatively larger pulses than single photon pulses, which would be selectively discriminated by a high discriminator level setting. These would occur preferentially in the earlier part of the decay when the emission is greatest. Thus, a high discriminator level setting will tend to distort the curve, giving greater weight to the earlier part of the curve.

In practice, with the level set high, accumulation of 100000 counts on a particular sample took ca. 9h, with a background of less than 10 counts per channel. On lowering the level, the lifetime obtained was lengthened slightly, the data was gathered more quickly, but the background rose to ca. 150 counts per channel.

Calibration was performed using a 16ns delay line. A very sharp 'decay' curve was produced by reflecting light into the stop PM; this was shifted by 40 channels (in the settings used for the compounds in this study) on inclusion of a 16ns delay, giving a channel spacing of 0.4ns per channel.

Output from the TAC was analysed (Northern Instruments Minecon NS633 256 channel analyser) and fed via punched tape into computer programs discussed later.

The instrumental response function (lamp curve) was obtained by either reflection, or by scattering (glycogen solution). The only parameters altered during the determination of the lamp curve were the sample, the excitation wavelength (high enough to allow transmission by the filter which was retained), and the slits which were slightly reduced to prevent 'pulse pile - up'.

Treatment of Data.

The processing of data for each compound measured involves two items, the instrumental response function (lamp curve, F(t)) and the curve obtained from the sample (the data curve, D(t)). Then, in principle, the observed data curve is given by[142]

$$D(t) = \int_0^t F(t - x).f(x).dx \qquad (2)$$

where f(t) is the true decay curve. There are several methods of extracting f(t) from the experimental curves, perhaps the most common being convolution of exponentials

$$f(t) = A \exp(-t/\tau) \qquad (3)$$

(of lifetimes chosen by trial and error) with the lamp curve to give the optimum fit with the data curve. A second method is the method of moments of Isenberg et. al.[144], in which moments, defined by

$$\mu_k = \int_0^\infty t^k F(t)\, dt \qquad (4)$$

$$m_k = \int_0^\infty t^k D(t)\, dt \qquad (5)$$

are evaluated, and these are used to calculate values of directly. The latter method has the advantage that no assumption of only one component is needed, - the number of components can be determined.

Both methods were used here; computer programs used are listed in the appendix. That for the convolution method is a modification of a program supplied by Applied Photophysics Ltd., and the moments program was written by the author, using the theory of Isenberg et. al.[144,145,146]

It is evident that when the lifetime is of similar magnitude to the width of the lamp curve, then the accuracy of the evaluated lifetime will be heavily dependent on the accuracy of the lamp curve. As Wahl et. al. have shown[147], an experimentally determined lamp curve is likely to be a distortion of the lamp curve actually applicable to the data. The instrumental response is made up primarily of two parts, that due to the lamp output, which may vary with wavelength, and that due to the PM, which may also vary with wavelength. It is impossible experimentally to determine lamp and data curves under identical wavelength conditions of excitation and emission, hence the abovementioned distortion. Wahl et. al.[147] circumvented this problem by using a reference compound of known lifetime. These authors deconvoluted the data curve for p - terphenyl against the predetermined lifetime, 0.96ns, using the relation

$$F(t) = D(t) + \tau \frac{dD(t)}{dt} \qquad (6)$$

Using this 'true' lamp curve, F(t), they were able to fit experimental data for N - acetyltryptophanamide 'perfectly' to a single exponential of 7.3ns.

This method, as presented, suffers from the disadvantage that it is necessary to know the lifetime of the reference compound with accuracy. However, literature values of lifetimes below 10ns rarely agree to better than 5%, often worse[142,148]. This approach may be modified slightly as is now described, in order to avoid this problem.

Assume that the lifetime of the reference compound is known approximately, but that the decay is a single exponential (i.e. the reference compound is chosen such that the assumption of a single exponential is valid). Then a lamp curve may be calculated by Wahl's method for the approximate lifetime. If now this is used to determine the lifetime of a second compound, also known to decay with a single exponential, then the fit (measured by the deviation of the calculated curve fron the experimental data curve) will only be perfect if the lamp curve is correct. Thus, by varying the lifetime around the approximate value, and testing the fit with a second compound, a value for the lifetime will be found which gives the best fit. This represents the correct value for the lifetime of the reference compound, and gives the correct lamp curve.

This method is still subject to wavelength errors occurring because of differences in the spectra of the various compounds used, and this error occurs in results quoted here. However, by using a second monochromator before the stop PM, it should be possible to minimise this. The restriction applies that only compounds which have similar spectral properties may be compared.

Using anthracene as a reference compound (lit. lifetimes 5.0ns/cyclohexane, 5.8ns/heptane[93,148]), lamp curves at intervals of 0.1ns were calculated (program LCALC, written here from Wahl's theory[147]), and these were used to evaluate the lifetime of indole in cyclohexane by iterative convolution. The best fit was found for a lifetime of 6.0ns for the anthracene (fig. 2).

This gave an indole lifetime of 9.0ns (lit. 9.1[30]), compared with 8.5ns (and a worse fit) using the experimental lamp curve.

The method of moments was also used. This method was found to be extremely sensitive to variations in the lamp curve. A 2 component analysis rarely worked, as the second component (which should be of negligible amplitude in a single decay system) was of a small, but not negligible amplitude, and this interfered with the accuracy of the first component. A single component analysis gave lifetimes comparable to those from the iterative convolution method, and these values are quoted below. Alteration of the figure used as the background (estimated from the counts in the first few channels), greatly affected the results by the moments method, but less those by iterative convolution. It is not clear whether the poor quality of results by the moments method is because of an inaccurate lamp curve, variation in the sample during data collection (resulting in a data curve of more than one component), or an inaccurate estimation of the background in both lamp and data curves. More reliance can be placed on the results obtained here by iterative convolution, since these were less subject to variation in parameters such as the background level.

Table 1 Fluorescence Lifetimes.

Compound	A	B	C	D	E	F
Indoline/c	2.0	1.8	2.2	2.7	2.2	1.9
5-methylindole/c	9.2	8.9	9.7	10.0	9.5	9.2
Anthracene/c	5.5	5.3	6.0	6.6	6.1	5.8
Indole/c	8.5	8.2	9.0	9.3	8.8	8.5
5-methylindole/m	4.4	4.3	4.9	5.2	4.7	4.4
Ethyl indoleacetate /m	0.6	0.5	0.7	2.0	1.6	1.5
Indole/m	3.9	3.7	4.3	4.6	4.2	3.8
Anthracene/c (repeat)	5.4	5.2	5.9	6.6	6.1	5.8

Key: A experimental lamp curve 1)
)
B experimental lamp curve 2) iterative convolution
)
C 6.0ns calculated curve)

D experimental lamp curve 1)
)
E experimental lamp curve 2) method of moments
)
F 6.0ns calculated curve)

Solvents: c = cyclohexane; m = methanol. Solutions were nitrogenated.

D. MISCELLANEOUS EXPERIMENTAL PROCEDURES

pK Values.

These were evaluated from titration curves obtained by conventional means using a Radiometer TTT2/ABU12 automatic titration system.

Conductance Measurements.

These were obtained on a Wayne - Kerr conductance bridge, using a thermostatted conductance cell at 25°.

I.R. Measurements.

Routine I.R.s were run on a Unicam SP200 spectrophotometer; a Grubb - Parsons Spectromaster was used for quantitative measurements.

Melting Points.

An Electrothermal apparatus was used; quoted values are uncorrected.

Half Wave Potentials.

A Cambridge Polarographic analyser, model 82P was used.

The technical assistance of Mr. C. Linskill is acknowledged in the operation of this instrument.

E. SYNTHETIC EXPERIMENTAL PROCEDURES.

Reaction of Ethyl Oxalyl Chloride with the Pyrrolidine Enamine of Cyclododecanone.

N - 1 - pyrrolidinyl cyclododecene (43.5g) was prepared from cyclododecane (36g) by the method of Stork et. al.[43]. This was immediately dissolved in dioxan (200ml), cooled in ice-water, and agitated whilst adding dropwise ethyl oxalyl chloride (40g) over 25min. After addition of ca. two thirds of the acid chloride, the yellow precipitate formed at first redissolved leaving a dark brown solution. This was stirred overnight at room temperature, then refluxed 1h with 10% HCl (50ml). After cooling and addition of water (500ml), ether extraction gave only cyclododecanone. The aqueous layer, on standing for 12 days, deposited large brown crystals. These were recrystallised from ethanol, yielding a bright yellow crystalline solid, m.p. 223 - 226°. A further yield of the same compound was obtained by making the aqueous solution alkaline. Total yield 12.7g.

I.R. (Nujol) 3400, 1730, 1640, 1550, 1540 K

U.V. (EtOH) λ_{max} 251, 358 nm. M^+ 289 ($C_{18}H_{27}NO_2$)

Analysis: $C_{18}H_{27}NO_2$ requires C 74.74, H 9.34, N 4.84 %

found C 73.23, H 9.27, N 4.48 %

Ethyl 11 - bromoundecanoate.

11 - bromoundecanoic acid (50g) was refluxed in EtOH (150ml) containing conc. H_2SO_4 (2ml) + KBr (ca. 0.3g) for 5¼h. Water (50ml) was added, the excess EtOH was distilled off and the liquid was extracted with ether. 44.2g (80%) of a light yellow oily product was obtained.

I.R. (film) 1735, 1185 K

Ethyl 11 - cyanoundecanoate.

To KCN (13g) in water (20ml) was added ethyl 11 - bromo - undecanoate (44.2g) in methanol (40ml), and the mixture was refluxed 36h. Ca. 20ml MeOH was then distilled off; water (15ml) was added, and after decanting off from the solid KBr present, the liquid was extracted with ether. The solvent was evaporated and the residue distilled, giving 21.9g product, b.p. 130 - 140°/0.5mm Hg.

I.R. (film) 1740, 1200, 2300(w) K

Sodium 11 - hydroxyundecanoate.

11 - bromoundecanoic acid (10g) was heated in 10% NaOH solution for 40min on a steam bath, and allowed to cool overnight. The solid product was filtered off and dried in vacuo. An essentially quantitative yield was obtained by salting out with NaCl.

11 - (3' - Indolyl)undecanoic acid.

Sodium 11 - hydroxyundecanoate (5.9g) and KOH (0.3g) were refluxed under water separation in p - cymene (70ml) for ca. 1h, when indole (3.0g) and freshly prepared nickel powder (0.2g, freshly dried from old Raney nickel) were added. The mixture was further refluxed for 24h, when black lumps were apparent in the mixture. After cooling overnight, the product was extracted into aq. alkali (ca. 150ml), which was washed with ether, and acidified. The solid produced was extracted into ether; on drying and evaporation, a low yield of a dark brown solid was produced, which was purified with

difficulty by recrystallisation from aq. EtOH. m.p. 86 - 92°

I.R. (KBr) 1120, 1700, 3400(m) K

U.V. (Hexane) λ_{max} 273, 279, 291 nm.

M.S. M^+ 301(4),130(13),117(26),98(21),84(17),83(15),73(19), 69(29),60(17),55(38),41(28).

L - 1,2,3,4 - tetrahydrocarboline - 3 - carboxylic acid.

L - tryptophan (10g), N H_2SO_4 (50ml), water (160ml) and 40% formaldehyde solution (50ml) were mixed and stood at room temperature. After 90min, the mixture was made just alkaline with .880 ammonia, and stood overnight. The precipitate produced was filtered off after adding acetone to aid its coagulation. The product was purified by dissolving in hot alcohol + ammonia, filtering, boiling off excess ammonia and cooling, when the product separated. M.p. 291 - 295°(dec)

I.R. (Nujol) 740, 1640, 3380 K

D - 1,2,3,4 - tetrahydrocarboline - 3 - carboxylic acid.

This was similarly prepared from D - tryptophan.

M.p. 287 - 292°(dec)

L - 1 - methyl - 1,2,3,4 - tetrahydrocarboline - 3 - carboxylic Acid.

L - tryptophan (5g) was dissolved in water (25ml) containing 0.1N H_2SO_4 (3ml). Acetaldehyde (5ml) was added and the mixture was stirred overnight at room temperature. The white solid product produced was recrystallised from ca. 600ml boiling water, giving 2.4g fine white needles, m.p. 306 - 308°(dec) (lit. 293°, 297° [69]).

I.R. (Nujol) 740, 750, 1640, 2500(m), 2670(m), 3300 K

1,2,3,4 - tetrahydrocarbazole - 3 - carboxylic acid.

4 - oxocylohexylcarboxylic acid (1.42g) and phenylhydrazine (1.13g) were refluxed for 1h in glacial acetic acid (40ml). The mixture was poured into water and the buff solid product formed was recrystallised from ether / 60 - 80° petroleum ether. Yield 2.24g white product, m.p. 196 - 198½°. (lit. 195° [85]).

I.R. (Nujol) 740, 750, 1695, 3400 K

Ethyl 1,2,3,4 - tetrahydrocarbazole - 3 - carboxylate.

4 - oxocyclohexylcarboxylic acid (1.42g) and phenylhydrazine hydrochloride (1.47g) were refluxed with conc. H_2SO_4 (3ml) in EtOH (40ml) for 90min. After cooling, the mixture was poured into water and thrice extracted with chloroform. After evaporation of the solvent, the resulting dark oil was triturated with petroleum ether, yielding a dark solid. This was purified by boiling with charcoal in ethyl acetate, filtering through kieselguhr and precipitating overnight with petroleum ether, following with a recrystallisation from benzene, giving 1.45g of a white solid product, m.p. 93½ - 94½°.

I.R. (Nujol) 740, 1190, 1705, 3400 K

4 - Amino - 4 - cyanocyclohexyl benzoate.

4 - oxocyclohexyl benzoate (10g)[55] was added with stirring to a mixture of NH_4Cl (5g), KCN (5g), water (10ml) and .880 ammonia (15ml), and the liquid was stirred 2 days at room temperature. Water was added; the liquid was extracted with chloroform, and the organic solution was dried ($MgSO_4$) and

evaporated, yielding a solid which was recrystallised from ether / petroleum ether. A white solid product (7.4g), m.p. 120 - 126° was obtained.

I.R. (Nujol) 720, 1180, 1710, 2250(w), 3330(m), 3400(m) K

Analysis: $C_{14}H_{16}N_2O_2$ requires C 68.8 , H 6.6 , N 11.5 %

found C 68.52, H 6.50, N 10.99 %

4 - Cyano - 4 -(benzoxycarbonylamino)cyclohexyl benzoate.

$NaHCO_3$ (1.68g) and 4 - amino - 4 - cyanocyclohexyl benzoate (2g) were stirred vigorously in water (10ml) at room temp. Benzyl chloroformate (1.5g) was added dropwise and the mixture was stirred for 1h. During this time, the suspension became successively a pasty mixture, semi - oil, pasty mixture, and finally a solid suspension. This was filtered off, washed with water and recrystallised from ethyl acetate / petroleum ether. Total yield of the product was 2.33g, m.p. 126 - 128°.

I.R. (Nujol) 720, 1180, 1540, 1695, 1703, 3300 K

4 - Acetoxycyclohexanone ethylene ketal.(VI)

4 - Acetoxycyclohexanone (50g)[57], ethanediol (60ml) and p - toluenesulphonic acid (1.0g) were refluxed in dry benzene (400ml) with vigorous stirring, under water separation. After 8h, 9 - 10ml water had collected (some ethanediol also came over), and the benzene was distilled off. The residual oil was taken up in chloroform, washed twice with water and dried with anhydrous K_2CO_3. The chloroform was removed under reduced pressure, yielding 51.3g product (80%). An analytical sample was prepared by distillation of this product, b.p. 94 - 96° / 1 - 2mm Hg; $n_D^{23.5°}$ 1.4642.

I.R. (film) 925, 1033, 1100, 1228, 1245, 1377, 1732 K

M.S. 43(71),86(42),87(28),91(33),99(100),140(51),155(15).

Analysis: $C_{10}H_{16}O_4$ requires C 59.98, H 8.05 %

found C 60.12, H 8.10 %

4 - Hydroxycyclohexanone ethylene ketal. (VII)

4 - acetoxycyclohexanone ethylene ketal (48.2g) was refluxed with KOH (24g) in methanol (250ml) containing water (30ml) for 4h. About $\frac{2}{3}$ of the methanol was then removed by distillation, the liquid was cooled and water (250ml) was added. The solution was extracted with chloroform (5 times, 50ml), the extracts were dried with anhydrous $MgSO_4$ and evaporated under reduced pressure, yielding 37.7g 4 - hydroxy-cyclohexanone ethylene ketal as a colourless oil (75% after distillation).

I.R. (film) 760, 930, 1035, 1105, 3450 K

By treatment with p - nitrobenzoyl chloride in the usual manner, this alcohol formed its p - nitrobenzoate as white crystals, m.p. $102\frac{1}{2}$ - $103\frac{1}{2}^{\circ}$.

I.R. (KBr) 720, 930, 1100, 1278, 1355, 1716 K

Analysis: $C_{15}H_{17}NO_6$ requires C 58.63, H 5.58, N 4.56 %

found C 58.52, H 5.42, N 4.56 %

4 - oxocyclohexanone monoethylene ketal. (IV)

Chromium trioxide (24g, 'Analar' reagent), vacuum dried at 80° overnight, was added with stirring to methylene dichloride (600ml, P_2O_5 dried) containing pyridine (37.96g, NaOH dried). The dark liquid was cooled to 0° whilst stirring for 20 min. 4 - hydroxycyclohexanone ethylene ketal (6.32g) was added in one portion, and the liquid was stirred

a further 20 min at 0°, when it was allowed to warm to room temperature. The dark liquid was decanted from the tarry residue, which was washed 4 times with ether. The combined organic solutions were washed with saturated aq. NaCl containing 5% NaOH (4 times), neutral saturated NaCl (4 times) then dried with anhydrous K_2CO_3. Evaporation under reduced pressure yielded an oil which crystallised on cooling. This was taken up in ether, the black solid impurities were filtered off, giving a pale yellow solution. Reduction of volume and addition of 60 - 80° petroleum ether to turbidity yielded on standing white crystals of the product (5.14g, 82%). Scaling up of this reaction gave lower yields. M.p. 71 - 73°.

I.R. (Nujol) 920, 1083, 1138, 1700 K

Analysis: $C_8H_{12}O_3$ requires C 61.51, H 7.74 %

found C 61.78, H 7.85 %

4 - Amino - 4 - cyanocyclohexanone ethylene ketal. (VIII)

4 - oxocyclohexanone monoethylene ketal (IV) (3.0g), NH_4Cl (3.0g), KCN (3.0g) and 0.880 ammonia solution (4.5ml) were stirred (4 days) in water (25ml) containing methanol (3ml). An equal volume of water was then added, and the solution was extracted with chloroform 4 times. The extract was dried ($MgSO_4$) and evaporated, the residue being recrystallised from ether/60 - 80° petroleum ether, yielding 2.45g pure product, with a further 0.7g second crop (total yield 87%). M.p. 39 - 50° (mixture of isomers).

I.R. (film) 940, 1040, 1110, 1165, 3300(m), 3370(m), 2250(w) K

Analysis: $C_9H_{14}N_2O_2$ requires C 59.32, H 7.74, N 15.37 %

found C 59.32, H 7.86, N 15.18 %

Spiro imidazoli - 2,5 -dione - 4,4' - cyclohexanone ethylene ketal. (IX)

The above aminonitrile (3.0g) was stirred 3 days with ammonium carbonate (10g) in water (15ml) at room temperature. The white solid produced was filtered off, washed with water and dried at 100° in vacuo, giving 3.37g product (90.5%), m.p. 245 - 247°.

I.R. (KBr) 1727, 1762, 1100(m), 3200(m) K

Analysis: $C_{10}H_{14}N_2O_4$ requires C 53.09, H 6.23, N 12.38 %

found C 53.22, H 6.26, N 12.34 %

4 - oxocyclohexane - 1 - amino - 1 - carboxylic acid ethylene ketal. (X)

The hydantoin (IX) (4.0g), and barium hydroxide octahydrate (15.4g) were heated at 150° in water (150ml) in a bomb for 6h. After cooling to room temperature, excess solid ammonium carbonate was added, and the mixture was heated on a water bath 30 min. This mixture was left at room temperature over - night, then the $BaCO_3$ produced was filtered off. The filtrate was taken to dryness in vacuo, and the residue was crystallised from aqueous methanol, yielding fine colourless needles of product, m.p. 301 - 304° (dec) (2.83g, 80%). This product was shown to be free of Ba^{2+} and NH_4^+ by usual tests.

I.R. (Nujol) 1560, 1620, 1100(m), 2200(m), 2600(m) K

Analysis: $C_9H_{15}NO_4$ requires C 55.72, H 7.51, N 6.96 %

found C 53.46 H 7.43, N 7.03 %

3 - Amino - 1,2,3,4 - tetrahydrocarbazole - 3 - carboxylic acid (XI)

To a solution of compound (X) (2.02g) in water (30ml) and

concentrated HCl (2ml), was added phenylhydrazine hydrochloride (1.46g), and the mixture was heated to 100° for 50min, then refluxed 5 min. After cooling to room temperature and neutralisation with 0.880 ammonia/water (3/1) to pH 7.2, copious precipitation occurred. The white plates were filtered off, washed with water then acetone. After drying at 100° in vacuo, the product, (1.77g, 77%) was obtained, dec ca. 300°.

I.R. (Nujol) 755, 1405, 1510, 1560, 1582, 1673, 3450(m) K

Analysis: $C_{13}H_{14}N_2O_2$ requires C 67.81, H 6.13, N 12.17 %

found C 67.92, H 6.15, N 11.95 %

N - 3 - Acetamido - 1,2,3,4 - tetrahydrocarbazole - 3 - carboxylic acid. (XII)

To the amino acid (XI) (1.00g) dissolved in 2M NaOH (20ml) was added acetic anhydride (5ml) at room temperature in 8 portions alternately with sufficient NaOH to keep the solution alkaline, with shaking between additions. A white precipitate was formed, apparently insoluble in NaOH solution. After cooling in ice/water 40 min, the solid was filtered and washed twice with a little water. The solid sodium salt was purified by dissolving in water, adding a few drops of dilute HCl to precipitate the free acid, which was filtered and dried, giving 0.68g white solid product, m.p. 278 - 281°(dec). The mother liquor deposited further crystals on standing, total yield 90%.

I.R. (Nujol) 740, 1555, 1610, 1720, 3350, 3380(m) K

Analysis: $C_{15}H_{16}N_2O_3$ requires C 66.16, H 5.92, N 10.29 %

found C 66.09, H 5.82, N 10.02 %

Cyclohexane - 1,4 - dione monoethylene ketal phenylhydrazone.

4 - oxocyclohexanone monoethylene ketal (IV) (6.0g), and phenylhydrazine (4.3g) were separately dissolved in minimal volumes of water, and the solutions were mixed. The resulting milky emulsion was extracted with ethyl acetate (5 times), the extract was dried (anhydrous $MgSO_4$), and evaporated under reduced pressure, to give a quantitative yield of the product which was not further purified.

I.R. (film) 700, 755, 1100, 1504, 1604, 3380(m) K

3 - oxo - 1,2,3,4 - tetrahydrocarbazole ethylene ketal.(XIV)

The above phenylhydrazone (9.4g) was dissolved in dry benzene (250ml), to which freshly fused and powdered zinc chloride (ca. 5g) was added. The mixture was refluxed 90min under a Dean and Stark apparatus. Most of the benzene was distilled off; excess 10% NaOH was added, and the liquid was extracted with ethyl acetate. The extract was washed with 5% NaOH, then water; dried (anhydrous $MgSO_4$) and evaporated, yielding a dark solid. Trituration with ether gave a light coloured solid which was washed with ether. The mother liquors on passing through a neutral alumina column, eluting with ether, yielded a further amount of white solid product. Total yield 6.43g (74%), m.p. 145½ - 147° (from ether/ petroleum ether).

I.R. (KBr) 745, 1060, 1100, 1110, 3400 K

M.S. 143(100), 144(13), 229(22).

Analysis: $C_{14}H_{15}NO_2$ requires C 73.34, H 6.59, N 6.11 %

found C 73.09, H 6.63, N 5.94 %

3 - Oxo - 1,2,3,4 - tetrahydrocarbazole.(XIII)

The above ethylene ketal (XIV) (6.03g), and p - toluene - sulphonic acid (1.4g) were refluxed in acetone (150ml) 4h. The acetone was distilled off, and the residue was taken up in ethyl acetate, washed thrice with $NaHCO_3$solution, once with water, dried (anhydrous $MgSO_4$) and taken to low volume. Addition of ether and petroleum ether (60 - 80°) yielded on scratching, 5.2g buff solid. This was purified by suspending in in dilute HCl containing a little methanol to facilitate wetting, warming to reflux point then cooling, extracting with ethyl acetate and passing through a neutral alumina column eluting with ether/ethyl acetate (1/1). To the eluate was added a little petroleum ether, and cooling to 0° for 2h yielded a white solid, which was washed with ether and petroleum ether.
M.p. 158 - 159° (lit.[59] 148 - 150°).
I.R. (Nujol) 750, 1703, 3390 K
M.S. 77(12),128(14),129(11),130(20),143(24),154(12),156(100), 157(70),185(86),186(14).

N - 4 - Morpholinyl cyclohex - 3 - ene - 1 - one ethylene ketal.(XVI)

4 - oxocyclohexanone monoethylene ketal (IV) (3.12g) was dissolved in dry benzene (120ml), morpholine (3.5g) was added, and the mixture was refluxed overnight under water separation. After 14h, ca. 0.3ml water had collected; the benzene was distilled off, leaving an oil which was not further purified.

I.R. (film) 900, 1120, 1205, 865(m), 1060(m), 1650(m) K

Decalin - 2,6 - dione monoethylene Ketal.(XIX)

i) The above enamine (XVI) was stirred in dry benzene (25ml), and methyl vinyl ketone (1.4g) in benzene(2ml) was added dropwise at room temperature. After stirring for 30 min, the solution was refluxed 3.5h. The benzene was removed under reduced pressure, methanol (25ml) and water (25ml) were added, and the mixture was refluxed overnight. Most of the methanol was distilled off, water was added, and the mixture thrice extracted with ether. The extracts were washed with acidified NaCl solution, dried ($MgSO_4$), and evaporated, yielding an amber oil, which was a mixture of isomers of the enone ketals (XVII).

I.R. (film) 1060, 1120, 1665, 1705 K

ii) The above enone ketal mixture in dry ether(100ml) was added dropwise over 10 min to liquid ammonia (300ml) containing lithium (2.0g). The mixture was stirred a further 5 min, then ammonium chloride (20g) was added in portions. The ammonia was allowed to evaporate, water (200ml) was added, the solution was thrice extracted with ether, the extracts were dried ($MgSO_4$) and evaporated, yielding as a yellow viscous oil, 6 - hydroxy - 2 - decalone ethylene ketal.(XVIII)

I.R. (film) 1040, 1095, 3450 K

M.S. 55(10),86(28),99(100),125(45),126(11),210(12).

iii) The hydroxy ketal was oxidised as described for compound (IV), using 12g CrO_3, 19.0g pyridine, 300ml CH_2Cl_2. The product was passed down a basic alumina column, eluting with ether, yielding the product as a colourless oil (1.55g).

I.R. (film) 1090, 1710 K

Decalin - 2,6 - dione monoethylene ketal phenylhydrazone.(XX)

Phenylhydrazine (0.8g) in a little methanol was added to compound XIX (1.55g) dissolved in methanol containing a little water. The mixture was warmed, water (20ml) was added, and the methanol was distilled off. A viscous yellow oil separated which was washed with water. The oil solidified on cooling to a waxy solid. This was taken up in ether. Trituration induced the crystallisation of a white solid product (310mg, 14%) m.p. 115 - 118°. The remaining material could not be induced to crystallise by usual means.

I.R. (Nujol) 690, 750, 1100, 1505, 1603, 3350(m) K

M.S. 39(23),41(32),42(17),43(15),45(12),51(11),53(15),55(27), 65(32),66(14),67(17),77(38),78(15),79(12),81(11),86(32),91(13), 92(31),93(82),94(14),99(100),106(16),125(26),300(85),301(20).

M^+ $C_{18}H_{21}N_2O_2$ requires 300.1847, found 300.1838.

5H - 6,6a,7,8,9,10,10a,11 - octahydro - 9 - oxo - benzo[b] - carbazole ethylene ketal. (XXI)

Freshly fused, finely powdered zinc chloride (ca. 0.5g) was added to the above phenylhydrazone (310mg) dissolved in dry benzene (30ml). After refluxing 90min, the mixture was left at room temperature 90min. The benzene was then removed under reduced pressure, 10% NaOH solution (15ml) was added, and the mixture was extracted with ethyl acetate. The organic layer was dried ($MgSO_4$), and evaporated, yielding a buff solid which was washed with ether, giving the product (100mg), m.p. 191 - 193°.

I.R. (Nujol) 740, 950, 3450, 1070(m), 1100(m), 1130(m) K

M.S. 32(33),36(14),39(14),41(22),42(16),43(36),44(25),55(21), 57(10),77(17),86(27),99(100),100(10),125(46),126(12),130(14), 140(31),143(89),144(15),153(14),154(36),167(19),168(34),169(13), 180(16),181(19),182(26),184(10),210(13),139(11),283(83),284(19).

M^+ $C_{18}H_{21}NO_2$ requires 283.1578, found 283.1572.

5H - 6,6a,7,8,9,10,10a,11 - octahydro - 9 - oxo - benzo[b] - carbazole. (XV)

A solution of ketal (XXI) (100mg) in acetone (10ml) containing 2N HCl (1ml) was refluxed 50 min. The acetone was then distilled off, until the volume reached ca. 5ml, when a white crystalline precipitate formed. The mixture was cooled to 0°, the solid was filtered off and washed with water, then dried in vacuo, giving the white solid product, m.p. 234 - 236°.

I.R. (Nujol) 740, 1703, 3330 K

M.S. 130(11),143(100),144(15),168(11),239(71),240(13)

M^+ $C_{16}H_{17}NO$ requires 239.1311, found 239.1310.

6a - α -, and 6a - β - indolo [2,3 - b] androstan - 14 - one (XXII)

5 - α or 5 - β androstan - 3,17 - dione (1.44g) was heated to reflux in ethanol (45ml). To the boiling solution was added phenylhydrazine hydrochloride (0.73g) in water (15ml), dropwise with stirring. After ca. 5 min, the solution became cloudy, and a solid precipitated. The mixture was allowed to cool whilst stirring for 10 min. The white solid product was filtered off, washed with water and dried in vacuo. Yields in each case were almost quantitative.

6a - α m.p. 286 - 289° (dec).

I.R. (Nujol) 745, 1730, 3400 K

M.S. 31(11),32(24),41(10),44(12),45(11),93(10),143(100),144(19), 361(45),362(12).

6a - β dec. 285° without melting.

I.R. (Nujol) 745, 1725, 3320 K

M.S. 31(56),32(31),41(12),43(12),44(11),45(42),46(12),130(30), 143(100),144(20),182(15),361(67),362(19).

Analysis: $C_{25}H_{31}NO$ requires C 83.06, H 8.64, N 3.87 %

found C 83.31, H 8.51, N 4.10 %

6a - α - indolo [2,3 - b] androstan - 14 - ol.(XXIII)

5 - α androstan - 17β- ol - 3 - one (1.0g) was dissolved in 95% ethanol (20ml), to which was added phenylhydrazine hydrochloride (1.0g) in water (15ml) with stirring and warming. The initially clear solution was refluxed 1h on a water bath, then cooled to room temperature. The white solid was filtered off, washed with water and dried, giving a quantitative yield of product, m.p. 240 - 243°.

I.R. (Nujol) 743, 1050, 3350(broad band) K

U.V. λ_{max} 275nm, shoulders 290, 295 nm.

3 - (2' - Methylindol - 3'- yl) - propanoic acid, ethyl ester.

4 - Acetylbutanoic acid (2.6g) and phenylhydrazine hydrochloride (2.9g) were refluxed with conc. H_2SO_4 (1ml) in absolute EtOH (40ml) for 2h. After cooling, the red liquid was decanted from the small amount of white solid present (NH_4Cl), and was poured into water and ether extracted. After washing with K_2CO_3 solution, drying ($MgSO_4$), and evaporating, a dark, very

viscous oil remained, which would not solidify.

I.R. (film) 750, 1170, 1720, 3400 K

3 - (2' - Methylindol - 3' - yl) propanoic acid.

The above ester (1.0g) was refluxed in 10% methanolic NaOH (20ml) containing water (20ml) for 45 min. The methanol was then distilled off after adding water (20ml). The aqueous solution was washed with ether, boiled with charcoal, filtered, and acidified with 20% HCl. The brownish solid produced was filtered off and recrystallised from benzene/ petroleum ether (60 - 80°), yielding a white amorphous solid product, m.p. 136 - 137°.

I.R. (KBr) 750, 1205, 1290, 1305, 1695, 3400, bands ca. 2700(s) K

3 - Acetoxy - 1,2,3,4 - tetrahydrocarbazole.

4 - Acetoxycyclohexanone ethylene ketal (VI) (2.0g) and phenylhydrazine hydrochloride (1.5g) were heated in 75% acetic acid (50ml) for 30 min., then cooled and poured into water (50ml). The mixture was extracted with ether, washed with alkali and water, then dried (K_2CO_3), and evaporated to low volume. Addition of petroleum ether (60 - 80°) caused the precipitation of a white solid product, which was recrystallised from ether / petroleum ether. M.p. 90 - 92°

I.R. (Nujol) 740, 1260, 1705, 3400 K

Methyl 1,2,3,4 - tetrahydrocarbazole - 3 - carboxylate.

1,2,3,4 - tetrahydrocarbazole - 3 - carboxylic acid (500mg) was dissolved in methanol (15ml), and 5 drops of conc. sulphuric acid were added. The mixture was refluxed 2h, then poured into an equal volume of water after cooling. Ether extraction and

workup in the usual manner yielded 310mg (58%) of a white solid product, m.p. $60\frac{1}{2}$ - $61\frac{1}{2}^{\circ}$.

I.R. (Nujol) 740, 1200, 1710, 3400 K

3 - Amino - 3 - cyano - 1,2,3,4 - tetrahydrocarbazole.

4 - amino - 4 - cyanocyclohexanone ethylene ketal (VIII) (800mg), was dissolved in 95% ethanol (3ml), to which was added conc. HCl (10ml) followed by phenylhydrazine hydrochloride (800mg) in water (10ml). This was kept on a steam bath for 15 min, then was cooled in an ice bath. The buff crystalline solid was filtered off, then dissolved in warm water. A few drops of 10% NaOH were added until there was no further white precipitate. The mixture was then ether extracted, the extracts were dried ($MgSO_4$), and petroleum ether ($60 - 80^{\circ}$) was added to turbidity. After crystallisation, 0.9g (95%) of a white amorphous solid product was obtained, m.p. 146 - 150° (dec 151°).

I.R. (KBr) 755, 2250(w), 3300(m), 3350(m), 3400 K

Analysis: $C_{13}H_{13}N_3$ requires C 73.91, H 6.20, N 19.89 %

found C 73.99, H 6.23, N 19.81 %

APPENDICES

LIST OF COMPOUNDS.

Compound	m.p. (lit. m.p.)	details. (refs to lit. preps)
Indole	51-52 (52[71])	Koch - Light. Recryst. water, sublimed.
1 - methylindole	b.p.74-76/.5 (133/26[70])	ref 70; dist. in vac. basic alumina column.
2 - methylindole	58-59 (61[71])	BDH, sublimed.
3 - methylindole	95-96½ (95[71])	BDH, sublimed.
5 - methylindole	58-59 (60[71] 56[83])	R.N.Emanuel. Used as received.
7 - methylindole	81-82 (85[71])	Fluka AG, used as recd.
2,3-dimethylindole	106-107(107-109[71])	Gift A; sublimed.
3 - ethylindole	35-36½(37,43[72])	ref 72; sublimed.
3-n-heptylindole	57½-58½(60[72])	Gift A; sublimed.
3-n-nonylindole	67-68	Gift A; sublimed.
Cyclopent b indole	100-103(108[74,75])	Gift A; sublimed.
Tetrahydrocarbazole	117-118(117-118, 113-114[82])	Gift A; sublimed.
Cyclohept b indole	144-145½(143[76])	Gift A; sublimed.
Cyclooct b indole	74½-75(72-74[81], 74½-76[84])	Gift A; sublimed.
Indoleacetic acid	167-169(165[71])	BDH Recryst. water.
Indolepropanoic acid	134-135½(134[71])	BDH Recryst. water.
Indolebutanoic acid	123-124(124[71])	BDH Recryst. water.
Indoleoctanoic acid	127-130	Gift A; used as recd.
Ethyl indoleacetate	38-39(42-43[86])	Pfaltz & Bauer. Recryst. aq. EtOH.
Methyl indolepropanoate	79-80½(79-80[71])	Esterification of acid; Recryst. Ether/pet.ether.

Methyl indolebutanoate	70-70½(73-74[71])	Aldrich. Recryst. ether/ pet.ether, aq. EtOH.
Indoleacetamide	151½-152½(150-1[71])	Fluka. Used as recd.
2-methylindoleacetic acid	198-200(197-199d[78])	ref. 78
2-methylindolepropanoic acid	136-137(139[77])	ref. 77
2-carboxycyclopent b indole	217-218d(215[73])	Gift B; used as recd.
3-hydroxy THC	151-152(152-154[59])	ref. 59
3-acetoxy THC	90-92	Exptl.
3-oxo THC, ethylene ketal XIV	145½-147	NC
3-carboxy THC	196-198½(195[85])	Exptl.
Methyl THC-3-carboxylate	60½-61½	NC
Ethyl THC-3-carboxylate	93½-94½	NC
L-Tryptophan	ca.272d(278,252,289[71])	BDH, as recd.
D-Tryptophan	ca.269d(275-282[71])	Koch light, as recd.
DL-Tryptophan	288-290d(278-82,293[71])	Phase Sep, as recd.
N-Acetyltryptophan	183-186(188[80])	ref.80
N-Acetyl-L-tryptophanamide	194½-196(192-3[71])	Phase Sep.Used as recd.
DL-Tryptophanol	53-54(b.p.180-5/.1[87])	Pfaltz & Bauer, used as received.
Alanyl-tryptophan	297-299d	Phase Sep, used as recd.
3-indolylcarbinol	91-92(158[71])	R.N.Emanuel. Recryst. ether/pet.ether.
3-hydroxyethylindole	56-57(59[71])	Gift.A; used as recd.
L - tetrahydrocarboline-3-carboxylic acid	310-313d(310[49])	Exptl.
D - " " " "	287-292d	Exptl.
1-methyl " " " "	306-308d(293,297[69])	ref. 69
3-amino-3-carboxy THC, XI	ca. 300d	NC
3-acetamido-3-carboxy THC,XII	278-281d	NC

Indolylbutanone	93-94(93-94[79])	ref. 79
3-oxo THC XIII	158-159(148-150[59])	Exptl.
ketone XV (see text)	234-236	NC
indolosteroid XXIIa	286-289d	NC
indolosteroid XXIIb	285d	NC
indolosteroid XXIII	240-243	NC
Yohimbine hydrochloride	ca.295d(302[71])	Aldrich; as recd.
Yohimbinic acid monohydrate	263-266d(265-9[71])	Aldrich; as recd.
indoline	b.p.123/ca.10(b.p.70½/2[71])	Koch Light; dist. in vacuo.
p - terphenyl	210½-212(213,209[71])	Koch Light S;as recd.
Anthracene	216-217(216.1[71])	BDH analyt. as recd.

Abbreviations,

recd = received; pet.ether = 60 - 80° petroleum ether;

Exptl = see experimental section; S = Scintillation grade;

NC = New compound, see text.

Gift A = Gift from Dr. A.Z. Britten.

Gift B = Gift from B. Lacoume.

DERIVATION OF THE FORM OF LOGNORMAL APPLICABLE TO SPECTRAL DATA.

The basic lognormal[100] is the distribution function obtained by distributing ln x in a normal (Gaussian) way, i.e. by replacing x' in the normal distribution function (eq.1),

$$dF = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{1}{2\sigma^2}(x' - \mu)^2\right] dx' \qquad (1)$$

with ln x. Then $dx'/dx = 1/x$, and dF becomes

$$dF = \frac{1}{x\sigma\sqrt{2\pi}} \exp\left[-\frac{1}{2\sigma^2}(\ln x - \mu)^2\right] dx \qquad (2)$$

Eq. 2 represents the normalised version; in order to allow for any size of curve, a factor A (= area of the curve) may be inserted, and by replacing x by (x - a), the curve may be positioned anywhere on the x axis. Thus in terms of a distribution of extinction $\epsilon(\tilde{\nu})$ with respect to wavenumber, eq. 2 now becomes:

$$\epsilon(\tilde{\nu}) = \frac{A}{(\tilde{\nu} - a)\sigma\sqrt{2\pi}} \exp\left(-\frac{1}{2\sigma^2}\left[\ln(\tilde{\nu} - a) - \mu\right]^2\right) \qquad (3)$$

This is the most general form - the 4 - parameter lognormal. The parameters A, a, σ, and μ are statistically useful, but for spectral purposes, the parameters ϵ_o, $\tilde{\nu}_o$, W and ρ are more appropriate, where these are defined in the text.

From eq. 3, the practical parameters can be calculated as follows.

Mode $\tilde{\nu}_o$

This occurs when $\frac{d\epsilon(\tilde{\nu})}{d(\tilde{\nu} - a)} = 0$

Differentiating eq. 3:

$$\frac{d\epsilon(\tilde{\nu})}{d(\tilde{\nu}-a)} = \frac{A}{\sigma\sqrt{2\pi}(\tilde{\nu}-a)^2} \exp\left(-\frac{1}{2\sigma^2}\left[\ln(\tilde{\nu}-a)-\mu\right]^2\right)\left(-1-\frac{1}{\sigma^2}\left[\ln(\tilde{\nu}-a)-\mu\right]\right)$$

$= 0$ when $(\bar{\nu} - a) \rightarrow \infty$ or when

$$\mu - \ln(\bar{\nu}_o - a) = \sigma^2$$

$$\therefore \quad (\bar{\nu}_o - a) = \exp(\mu - \sigma^2)$$

$$\underline{\bar{\nu}_o = a + \exp(\mu - \sigma^2)} \qquad (4)$$

Modal Value ϵ_o

Substituting $\bar{\nu} = \bar{\nu}_o$ in eq. 3, using eq. 4,

$$\epsilon_o = \frac{A}{\exp(\mu - \sigma^2)\sigma\sqrt{2\pi}} \exp\left(-\frac{1}{2\sigma^2}(\mu - \sigma^2 - \mu)^2\right)$$

$$= \frac{A \exp(\sigma^2 - \mu)}{\sigma\sqrt{2\pi}} \exp(-\sigma^2/2)$$

$$\underline{\epsilon_o = \frac{A}{\sigma\sqrt{2\pi}} \exp(\sigma^2/2 - \mu)} \qquad (5)$$

Width at half height, W

At $\epsilon(\bar{\nu}) = \frac{1}{2}\epsilon_o$, $(\bar{\nu} - a)$, denoted by x, is given by: (using eqs. 3 and 5)

$$\frac{A}{2\sigma\sqrt{2\pi}} \exp\left\{\frac{\sigma^2}{2} - \mu\right\} = \frac{A}{x\sigma\sqrt{2\pi}} \exp\left(-\frac{1}{2\sigma^2}\left[\ln x - \mu\right]^2\right)$$

$$\frac{1}{2} \exp\left(\frac{\sigma^2}{2} - \mu\right) = \frac{1}{x} \exp\left(-\frac{1}{2\sigma^2}\left[\ln x - \mu\right]^2\right)$$

taking natural logarithms,

$$\ln(\tfrac{1}{2}) + (\sigma^2/2 - \mu) = -\ln x - \frac{1}{2\sigma^2}\left[\ln x - \mu\right]^2$$

$$= -\ln x - (\mu^2/2\sigma^2) - \frac{(\ln x)^2}{2\sigma^2} + \frac{\ln x}{\sigma^2}$$

Putting $\ln x = X$, $p = \sigma^2 - \mu$, $q = 2\ln 2$, then

$$\frac{X^2}{\sigma^2} + \frac{2pX}{\sigma^2} + p - q + \frac{\mu^2}{\sigma^2} - \mu = 0$$

$$X = \frac{-2p \pm \sqrt{4p^2 - 4p^2 + 4q\sigma^2}}{2}$$

$$= -p \pm \sigma q$$

i.e. $\ln x = \mu - \sigma^2 \pm \sigma\sqrt{2 \ln 2}$

$$x = \exp(\mu - \sigma^2) \exp(\pm\sigma\sqrt{2 \ln 2})$$

W is the difference between these two values of x.

$$W = \exp(\mu - \sigma^2) \cdot \left[\exp(\sigma\sqrt{2 \ln 2}) - \exp(-\sigma\sqrt{2 \ln 2})\right]$$

$$\text{or, } W = \exp(\mu - \sigma^2) \frac{\exp(\sigma\sqrt{2 \ln 2})^2 - 1}{\exp(\sigma\sqrt{2 \ln 2})} \qquad (6)$$

Skewness

From the definition of ρ, using the expressions for x above and eq. 4,

$$\rho = \frac{\exp(\mu - \sigma^2) \exp(\sigma\sqrt{2 \ln 2}) - \exp(\mu - \sigma^2)}{\exp(\mu - \sigma^2) - \exp(\mu - \sigma^2) \exp(-\sigma\sqrt{2 \ln 2})}$$

$$= \frac{\exp(\sigma\sqrt{2 \ln 2}) - 1}{1 - \exp(-\sigma\sqrt{2 \ln 2})}$$

$$\rho = \exp(\sigma\sqrt{2 \ln 2}) \qquad (7)$$

Eqs. 4, 5, 6 and 7 may now be used to rewrite eq 3 in terms of the spectral parameters.

Denoting σ by c and $\exp\mu$ by b, then from eq. 7,

$$\rho = \exp(c\sqrt{2 \ln 2}) \quad \text{or}$$

$$c = \ln\rho / \sqrt{2 \ln 2} \qquad (8)$$

From eq. 6,

$$W = b \exp(-c^2) \cdot \frac{(\rho^2 - 1)}{\rho}$$

$$b = \frac{W\rho \exp(c^2)}{\rho^2 - 1} \qquad (9)$$

From eq. 4,

$$\bar{\nu}_o = a + b \exp(-c^2)$$

$$= a + \frac{W\rho}{\rho^2 - 1}$$

$$a = \bar{\nu}_o - \frac{W\rho}{\rho^2 - 1} \qquad (10)$$

From eq. 5,

$$\epsilon_o = \frac{A}{c\sqrt{2\pi}} \exp(c^2/2). \; 1/b$$

$$A = \epsilon_o \, b \, c \sqrt{2\pi} \exp(-c^2/2) \qquad (11)$$

Hence, substituting into eq. 3,

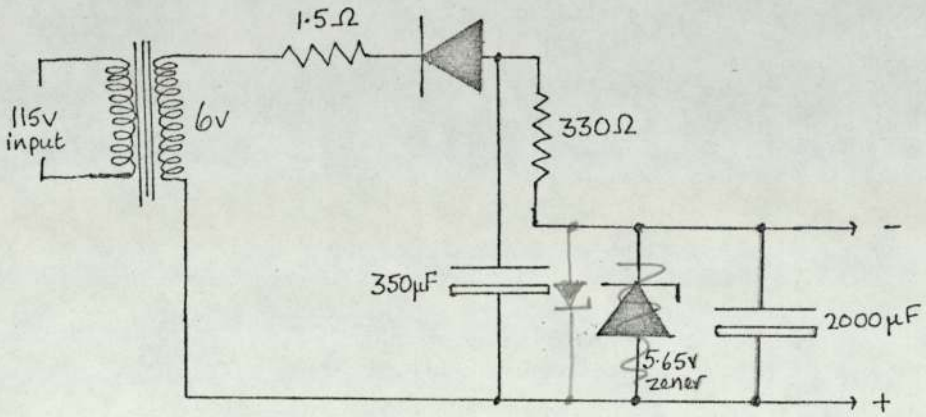
$$\epsilon(\bar{\nu}) = \frac{\epsilon_o \, b}{(\bar{\nu} - a)} \exp(-c^2/2). \exp\left(- \frac{1}{2c^2} \left[\ln \frac{\bar{\nu} - a}{b} \right]^2 \right)$$

where $c = \ln\rho / (2 \ln 2)^{\frac{1}{2}}$

$b = W\rho \exp(c^2) / (\rho^2 - 1)$ (12)

and $a = \bar{\nu}_o - W\rho/(\rho^2 - 1)$

QED



RECORDER BIAS SUPPLY FOR AMINCO - BOWMAN SPECTROPHOTOFLUORIMETER.

COMPUTER PROGRAMS

All programs are in standard ICL Algol or Fortran, to run on the ICL 1905E computer under the George 3 operating system.

LINEUP

- Program to carry out the spectral subtraction technique, chap. 3. The data are the 2 spectra, presented on channels TR1 and TR2 as lists of 300 numbers in free format; the variables F and D in the program correspond to the ^1L_b and ^1L_a curves output, respectively. L is the number of points by which spectrum B must be displaced to align the 0 - 0 peaks with spectrum A. This is estimated visually, as is K, the separation of the ^1L_a peaks. The program allows the calculation of each component for several values of K; the best one can be selected visually. ORD allows the subtraction to be performed in the correct order.

LOGNORMFIT

This program was written in purely functional form and is not easily readable. Since programs have been written by other authors, which are capable of fitting this equation, (e.g. 'Programs for Resolution of Spectra using Lognormal Curves' by Siano et. al., from Dr. D.E. Metzler, Iowa State University), it is not reproduced here.

LINEUP / 1

```
 0 'PROGRAM'(AXXX)
 1 'INPUT'0=CR0
 2 'INPUT'1=TR1
 3 'INPUT'2=TR2
 4 'OUTPUT'0=LP0
 5 'OUTPUT'1=LP1
 6 'COMPACTDATA'
 7 'COMPACT'
 8
 9    'BEGIN'
10       'ARRAY' A[1:300],B[1:300],C[1:300],D[1:300],F[1:300];
11       'INTEGER' I,J,K,ORD,L;
12        'FOR'I:=1'STEP'1'UNTIL'300'DO'
13        A[I]:=B[I]:=C[I]:=D[I]:=F[I]:=0;
14        L:=READ;
15        SELECT INPUT(1);
16        'FOR'I:=1'STEP'1'UNTIL'300'DO'
17        A[I]:=READ;
18        SELECT INPUT(2);
19        'FOR'I:=1'STEP'1'UNTIL'300-L'DO'
20        B[I+L]:=READ;
21        SELECT INPUT(0);
22        K:=READ;
23        ORD:=READ;
24        'IF'ORD=0'THEN'   'BEGIN'
25        'FOR'I:=1'STEP'1'UNTIL'300'DO'
26        C[I]:=A[I]-B[I];
27        'END''ELSE' 'BEGIN'
28        'FOR'I:=1'STEP'1'UNTIL'300'DO'  C[I]:=B[I]-A[I];
29        'END';
30        SELECT OUTPUT(0);
31      WRITETEXT('('%%F[1-60]%%%D[1-60]%%%F[-120]%%%
32     D[-120]%%%F[-180]%%%D[-180]%%%F[-240]%%%D[-240]%%%
33       F[-300]%%%D[-300]%')');
34        NEWLINE(1);
35 L1:     WRITETEXT('('K%=')'); PRINT(K,4,0);
36        WRITETEXT('('%%%L%=')'); PRINT(L,4,0);
37       NEWLINE(1);
38    'FOR'I:=1'STEP'1'UNTIL'300'DO'
39         'BEGIN'
40           J:=-1;
41           'FOR'J:=J+1'WHILE'(I-J*K)>0'DO'
42           D[I]:=D[I]+C[I-J*K];
43         'IF'ORD=0'THEN'F[I]:=A[I]-D[I]
44          'ELSE'F[I]:=B[I]-D[I];
45        'END';
46           SELECT OUTPUT(0);
47        'FOR'I:=1'STEP'1'UNTIL'60'DO'
48        'BEGIN'
49 PRINT(F[I],4,2); PRINT(D[I],4,2);
10.17.37←
```

LINEUP / 2

```
    50   PRINT(F[60+I],4,2); PRINT(D[60+I],4,2);
    51    PRINT(F[120+I],4,2); PRINT(D[120+I],4,2);
    52    PRINT(F[180+I],4,2); PRINT(D[180+I],4,2);
    53   PRINT(F[240+I],4,2); PRINT(D[240+I],4,2); NEWLINE(1);
    54     'END';
    55          SELECT OUTPUT(1);
    56    'FOR'I:=1'STEP'1'UNTIL'300'DO' 'BEGIN'
    57          PRINT(F[I],5,5);  NEWLINE(1)  ; 'END';
    58      I:=READ;
    59        'IF'I=0'THEN''GOTO'LZ;
    60        'FOR'I:=1'STEP'1'UNTIL'300'DO'
    61        D[I]:=F[I]:=0;
    62        SELECT INPUT(0);
    63        K:=READ;
    64        SELECT OUTPUT(1);    NEWLINE(20);
    65        SELECT OUTPUT(0);    NEWLINE(20);
    66        'GOTO'L1;
    67     LZ:  'END'  OF PROGRAM;
    68
10.21.04←
```

CORRCALC

```
    'BEGIN'
     'REAL'S,T,ABS,X,D,UP,LO,C,A,B,Y,HG,G,SA,SB,U,V,CF;
     WRITETEXT('('ABSORBANCE%%OUTER%POS%%%OUTER%NEG%%%%INNER%%%%%%
       EXP[-1.15ABS]%CORRECTION%FACTOR')');
     NEWLINE(2);
     'FOR'ABS:=0.05'STEP'0.05'UNTIL'1.5'DO'
      'BEGIN'
       S:=T:=0;
       D:=EXP(0.02*0.230259*ABS);
       'FOR'X:=-1.0'STEP'0.05'UNTIL'1.0'DO'
        'BEGIN'
        UP:=(39.35-2*X)/33.1
        ; LO:=(X+5.15)/33.1;
        C:=EXP(-0.230259*ABS*LO);
        A:=39.35-2*X;
        B:=506.55-55.9*X;
        Y:=LO;
L1:     HG:=C;
        C:=C/D;
        G:=((A-33.1*Y)*HG)/B;
        S:=S+G;
        Y:=Y+0.02;
        'IF'Y>UP'THEN''GOTO'L2'ELSE''GOTO'L1;
L2:     'END';
      S:=S/6283.174;
      'FOR'X:=-1'STEP'0.05'UNTIL'1'DO'
      'BEGIN'
        UP:=-(X+5.15)/33.1
    ;    LO:=(2*X-39.35)/33.1;
        C:=EXP(-0.230259*ABS*LO);
        A:=39.35-2*X;
        B:=506.55-55.9*X;
        Y:=LO;
L3:    HG:=C;     C:=C/D;
        G:=((A+33.1*Y)*HG)/B;
        T:=T+G;
        Y:=Y+0.02;
        'IF'Y>UP'THEN''GOTO'L4'ELSE''GOTO'L3;
L4:     'END';
       T:=T/6283.174;
     SA:=EXP(0.00696*ABS);
     SA:=(SA-1/SA)/2;
     SB:=EXP(0.03587*ABS);
     SB:=(SB-1/SB)/2;
      U:=27.02*SA*SB/(ABS*ABS);
      V:=EXP(-1.151295*ABS);
         CF:=(S+T+U)*V;
      PRINT(ABS,3,7);    PRINT(S,3,7);
     PRINT(T,3,7);       PRINT(U,3,7);
      PRINT(V,3,7);    PRINT(CF,3,7);
      NEWLINE(1);
     'END';      'END';
```

Results from CORRCALC

ABSORBANCE	OUTER POS.	OUTER NEG.	INNER	EXP (-1.15ABS)	C.F.
0.0500000	0.0112740	0.0111861	0.0067457	0.9440608	0.0213721
0.1000000	0.0112103	0.0112508	0.0067457	0.8912507	0.0200306
0.1500000	0.0111470	0.0113160	0.0067457	0.8413948	0.0243761
0.2000000	0.0110842	0.0113816	0.0067457	0.7943278	0.0256036
0.2500000	0.0110218	0.0114478	0.0067458	0.7498937	0.0219084
0.3000000	0.0109599	0.0115143	0.0067458	0.7079423	0.0206862
0.3500000	0.0108984	0.0115814	0.0067459	0.6683433	0.0192328
0.4000000	0.0108375	0.0116490	0.0067459	0.6309567	0.0184442
0.4500000	0.0107766	0.0117170	0.0067460	0.5956615	0.0174169
0.5000000	0.0107164	0.0117855	0.0067461	0.5623406	0.0164473
0.5500000	0.0106566	0.0118546	0.0067461	0.5308857	0.0155322
0.6000000	0.0105972	0.0119241	0.0067462	0.5011865	0.0146685
0.6500000	0.0105385	0.0119941	0.0067463	0.4731305	0.0138532
0.7000000	0.0104797	0.0120647	0.0067464	0.4466828	0.0130837
0.7500000	0.0104215	0.0121357	0.0067465	0.4216957	0.0123573
0.8000000	0.0103638	0.0122073	0.0067466	0.3981004	0.0116716
0.8500000	0.0103065	0.0122794	0.0067468	0.3758566	0.0110245
0.9000000	0.0102495	0.0123520	0.0067469	0.3548126	0.0104132
0.9500000	0.0101930	0.0124252	0.0067470	0.3349047	0.0098365
1.0000000	0.0101368	0.0124989	0.0067472	0.3162270	0.0092917
1.0500000	0.0100810	0.0125731	0.0067473	0.2985575	0.0087774
1.1000000	0.0100256	0.0126479	0.0067475	0.2818375	0.0082920
1.1500000	0.0099706	0.0127232	0.0067477	0.2660718	0.0078336
1.2000000	0.0099160	0.0127991	0.0067478	0.2511879	0.0074007
1.2500000	0.0098618	0.0128755	0.0067480	0.2371506	0.0069921
1.3000000	0.0098079	0.0129525	0.0067482	0.2238714	0.0066061
1.3500000	0.0097544	0.0130301	0.0067484	0.2113482	0.0062417
1.4000000	0.0097012	0.0131082	0.0067486	0.1995255	0.0058976
1.4500000	0.0096485	0.0131870	0.0067488	0.1883642	0.0055726

FLUCORR.

This program corrects fluorescence spectra for the instrumental response with wavelength. The correction values are kept on a file which is assigned to CR1. The spectrum to be corrected is input on CR0 in free format, preceded by the start wavelength and the increment. The data is terminated by a negative number.

```
'PROGRAM'(FSP6)
'INPUT'0=CR0
'INPUT'1=CR1
'INPUT' 2 = TR0
'OUTPUT'0=LP0
'OUTPUT' 1 = LP1
'COMPACTDATA'
'COMPACT'
     'BEGIN'
      'BOOLEAN''PROCEDURE' TEST(N);
        'VALUE' N;
        'INTEGER' N;
      'EXTERNAL';
        'REAL''ARRAY' CORR[1:351];
        'REAL' SA,SB,SC,SD,L,F,OBS,FC,FCE,FCQ,TAST;
        'INTEGER' I,INIT,DX;
        SELECT INPUT(1);
        'FOR' I:=1 'STEP' 1 'UNTIL' 351 'DO'  CORR[I]:=READ;
      'IF' TEST(1) 'THEN ' SELECT INPUT (2) 'ELSE' SELECT INPUT(0);
      L1: SA:=SB:=SC:=SD:=0;
        PAPERTHROW;
        COPYTEXT ('('FINISH')');
        NEWLINE(2);
        WRITETEXT('('WAVELENGTH%%%WAVENUMBER%%OBSERVED'('12S')'C%O%R
          R%E%C%T%E%D%%%%V%A%L%U%E%S'('1C26S')'SPECTRAL'('8S')'
         SPECTRAL'('10S')'PHOTON%SPECTRAL%%PHOTON%SPECTRAL'('
         1C2S')'[NM.]'('8S')'[KK.]'('5S')'IRRADIANCE'('6S')'
        IRRADIANCE'('8S')'IRRADIANCE'('6S')'IRRADIANCE'('1C42S'
         )'E[LAMBDA]'('8S')'EQ[LAMBDA]'('7S')'EQ[NU-BAR]')');
        NEWLINE(2);
        INIT:=READ;
         DX:=READ;
        L:=INIT;        I:=INIT-249;
      L2: F:=(1/L)*10000;
        OBS:=READ;
        'IF'OBS<0'THEN''GOTO'L3;
        'IF'L>600'THEN''GOTO'L4;
        FC:=OBS*CORR[I];
        SA:=SA+OBS*DX;
```

```
      SB:=SB+FC*DX;
      FCE:=FC*350.0/L;
      FCQ:=FC*L*L/122500.0;
      SC:=SC+FCE*DX;
      SD:=SD+FCQ*DX;                          FLUCORR/2
      SPACE(1);
      PRINT(L,4,2);
      SPACE(1);
      PRINT(F,4,4);
      SPACE(1);
      PRINT(OBS,4,2);
      SPACE(6);
      PRINT(FCE,4,3);
      SPACE(7);
      PRINT(FC,4,3);
      SPACE(6);
      PRINT(FCQ,4,3);
      NEWLINE(1);

    'IF'TEST(2) 'THEN'
     'BEGIN'
      SELECT OUTPUT (1);
      'IF' TEST(3) 'THEN'PRINT(FCQ,4,3) 'ELSE'PRINT(FC,4,3);
      NEWLINE(1);
      SELECT OUTPUT (0);
     'END';
      L:=L+DX;
      I:=I+DX;
      'GOTO'L2;
    L3: NEWLINE(1);
      WRITETEXT('('RELATIVE%AREAS')');
      SPACE(9);
      PRINT(SA,6,3);
      SPACE(3);
      PRINT(SC,6,3);
      SPACE(5);
      PRINT(SB,6,3);
      SPACE(5);
      PRINT(SD,6,3);
      TAST:=READ;
      'IF'TAST<0'THEN''GOTO'L1;
      'GOTO'L5;
    L4: 'BEGIN'
       NEWLINE(1);
      WRITETEXT('('YOU%HAVE%EXCEEDED%THE%CAPACITY%OF%THIS%
       PROGRAM%BY%IMPLYING%A%WAVELENGTH%GREATER%THAN%600%NM.')');
       NEWLINE(1);
       L6:
      TAST:=READ;
      'IF'TAST<0'THEN''GOTO'L3;
       'GOTO'L6;
       'END';
    L5: 'END';
```

DECONV

This program deconvolutes a spectral curve (presented as a series of intensity values at a constant wavelength interval) against a 3.5nm bandwidth (triangular slit function). The method used closely follows that of Jones and Venkataragharan[153], and the program is therefore not listed.

CONVOLUTE

This program reads in a band function (TRO) and convolutes it point by point with a spectrum input on CRO.

```
'PROGRAM'(AXXX)
'INPUT'0=CRO
'INPUT'3=TRO
'OUTPUT'0=LP0
'OUTPUT'4=LP1
'COMPACTDATA'
'COMPACT'
*****
    'BEGIN'
      'INTEGER'I;
       I:=READ;
        'BEGIN'
        'REAL' S,NO,P;
        'ARRAY' A[1:I];
        'INTEGER'J;
        S:=0;
        SELECT INPUT(3);
        P:=0;
        'FOR' J:=1'STEP'1'UNTIL' I 'DO'
          'BEGIN'
          A[J]:=READ;
          S:=S+A[J];
          'END';
         SELECT INPUT(0);
        L1:  COPYTEXT('('FINISH')');
        'FOR'J:=1'STEP'1'UNTIL'I'DO'
          'BEGIN'
          NO:=READ;
          P:=NO*A[J] + P;
          'END';
          P:=P/S;
        WRITETEXT('('CONVOLUTED%PRODUCT%IS:%%')');
        PRINT(P,5,5);
        NEWLINE(10);
        NO:=READ;
       'IF'NO<0'THEN''GOTO'L1;
      'END';
   'END'OF PROGRAM;
```

MOMENT

Evaluates lifetimes by the method of moments

```
0         LIST(LP)
1         LIBRARY(ED,SUBGROUPONE)
2         PROGRAM(GLGL)
3         INPUT 1 = CR0
4         INPUT 2 = TR1
5         INPUT 3 = TR2
6         OUTPUT 4 = LP0
7         COMPACT
8         TRACE 0
9         END
10        MASTER
11        COMMON D(256),F(256),NCH
12        READ(1,5)NCH,TCH,DBACK,FBACK,DEP,MD,N
13       5 FORMAT(I0,4F0.0,2I0)
14  C      NCH = NO. OF CHANNELS;  TCH = TIME INCREMENT PER CHANNEL;
15  C      DBACK = DATA BACKGROUND;  FBACK = LAMP BACKGROUND;
16  C      DEP = EXPONENTIAL DEPRESSION = (LAST DATA CHANNEL REQD/OBS.)
17  C          (0.5 FOR MINIMUM ERROR, 0.1 ACCEPTABLE ERROR)
18  C      MD = MOMENT DEPRESSION (0,1, OR 2);  N= NO. OF COMPONENTS .LE.4
19  C
20  C
21        READ(2,7)FST,(F(I),I=1,NCH)
22        READ(3,7)DST,(D(I),I=1,NCH)
23       7 FORMAT(256F0.0)
24  C
25  C     BACKGROUND CORRECTION
26  C
27        DO 10 I=1,NCH
28        F(I)=F(I)-FBACK
29        IF(F(I).LE.0.0)F(I)=0.0
30        D(I)=D(I)-DBACK
31        IF(D(I).LE.0.0)D(I)=0.0
32       10 CONTINUE
33  C     OLDELTA IS ARRAY OF CUTOFF CORRECTIONS
34        DIMENSION OLDELTA(10)
35        DO 15 I=1,10
36       15 OLDELTA(I)=0.0
37  C
38  C
39  C
40  C
41  C     EXPONENTIAL DEPRESSION
42  C
43        IF(DEP.EQ.1.0) GO TO 40
44        DEP = (ALOG(1.0/DEP))/(NCH*TCH)
45        DEPA = EXP(-DEP*TCH)
46        DEPB = DEPA
47        DO 30 I=1,NCH
48        F(I)=F(I)*DEPB
49        D(I)=D(I)*DEPB
50        DEPB = DEPB*DEPA
```

```
      30 CONTINUE
      40 CONTINUE
C
C     MOMENT CALCULATION - LAMP
C
         DIMENSION FMOM(10),XINT(256)
         M=2*N+1
         DO 100 K=1,M
         T=TCH
         DO 50 I=1,NCH
         XINT(I)=(T**K)*F(I)
      50 T=T+TCH
         L=K+1
         CALL SIMP(XINT,TCH,NCH,FMOM(L))
     100 CONTINUE
         CALL SIMP(F,TCH,NCH,FMOM(1))
         ANORM = 1000.0/FMOM(1)
         FMOM(1)=1000.0
         DO 110 I=2,M
     110 FMOM(I)=FMOM(I)*ANORM
C
         BNORM=ANORM
C
C     MOMENT CALCULATION - DATA
C
         DIMENSION DMOM(10)
         DO 200 K=1,M
         T=TCH
         DO 150 I=1,NCH
         XINT(I)=(T**K)*D(I)
     150 T=T+TCH
         L=K+1
         CALL SIMP(XINT,TCH,NCH,DMOM(L))
     200 CONTINUE
         CALL SIMP(D,TCH,NCH,DMOM(1))
         ANORM=1000.0/DMOM(1)
         DMOM(1)=1000.0
         DO 210 I=2,M
     210 DMOM(I)=DMOM(I)*ANORM
         WRITE(4,120)ANORM,BNORM
     120 FORMAT( ' NORMALISATION FACTORS-DATA:',F12.5,
        1   ' LAMP: ',F12.5)
          WRITE(4,121)
     121 FORMAT(///)
C
C    CALCULATION OF G VALUES
C
          DIMENSION G(10)
     300 G(1)=DMOM(1)/FMOM(1)
         DO 350 K=2,M
         K1=K-1
         SUM = 0.0
         DO 310 I=1,K1
         KI=K-I
         KII=KI+1
     310 SUM = SUM+G(I)*FMOM(KII)/FAC(KI)
     350 G(K)=((DMOM(K)/FAC(K1))-SUM)/FMOM(1)
```

```
108 C
109 C    POLYNOMIAL MATRIX - BY MOMENT INDEX DISPLACEMENT
110 C
111         DIMENSION GMAT(5,4)
112         NN=N+1
113         KI = MD
114         DO 400 I=1,NN
115         DO 380 J=1,N
116         KI=KI+1
117     380 GMAT(I,J)=G(KI)
118         KI=MD+I
119     400 CONTINUE
120 C
121 C   SOLVE FOR TAU
122 C
123         DIMENSION TAU(4),A(5),CONST(4),PL(2),PR(4),RI(4),INAC(4)
124         INTEGER IMAX
125         IF(N.EQ.1)GO TO 410
126         IF(N.EQ.2)GO TO 420
127         IF (N.EQ.3)GO TO 440
128         IF (N.EQ.4)GO TO 460
129         WRITE(4,405)
130     405 FORMAT(/,' ERROR: N MUST BE BETWEEN 1 AND 4' )
131         STOP
132     410 TAU(1)=GMAT(2,1)/GMAT(1,1)
133         GO TO 550
134     420 A(1)=GMAT(2,1)*GMAT(3,2) - GMAT(3,1)*GMAT(2,2)
135         A(2)=GMAT(3,1)*GMAT(1,2) - GMAT(1,1)*GMAT(3,2)
136         A(3)=GMAT(1,1)*GMAT(2,2) - GMAT(1,2)*GMAT(2,1)
137         RI(1)=A(2)*A(2) - 4.0*A(3)*A(1)
138         IF(RI(1).GT.0.0) GO TO 430
139         IF(RI(1).LT.0.0) WRITE(4,425)
140     425 FORMAT('  COMPLEX VALUE FOR TAU ')
141         IF(RI(1).EQ.0.0) GO TO 431
142         STOP
143     430 RI(1)=SQRT(RI(1))
144     431 TAU(1)=(RI(1) - A(2))/(2.0*A(3))
145         TAU(2)=(-A(2)-RI(1))/(2.0*A(3))
146         GO TO 550
147     440 RI(1)=GMAT(1,2)*GMAT(2,3) - GMAT(1,3)*GMAT(2,2)
148         RI(2)=GMAT(1,2)*GMAT(3,3) - GMAT(1,3)*GMAT(3,2)
149         RI(3)=GMAT(1,2)*GMAT(4,3) - GMAT(1,2)*GMAT(4,2)
150         RI(4)=GMAT(2,2)*GMAT(3,3) - GMAT(2,3)*GMAT(3,2)
151         PR(1)=GMAT(2,2)*GMAT(4,3) - GMAT(2,3)*GMAT(4,2)
152         PR(2)=GMAT(3,2)*GMAT(4,3) - GMAT(3,3)*GMAT(4,2)
153         A(1)=GMAT(2,1)*PR(2) - GMAT(3,1)*PR(1)+GMAT(4,1)*RI(4)
154         A(2)=-(GMAT(1,1)*PR(2)-GMAT(3,1)*RI(3)+GMAT(4,1)*RI(2))
155         A(3)=GMAT(1,1)*PR(1)-GMAT(2,1)*RI(3)+GMAT(4,1)*RI(1)
156         A(4)=GMAT(2,1)*RI(2)-GMAT(1,1)*RI(4)-GMAT(3,1)*RI(1)
157         DOUBLE PRECISION B(24)
158         PL(1)=00.0
```

```
      PL(2)=0.0
      CONST(1)=1.0E-16
      CONST(2)=1.0E-13
      CONST(3)=1.62
      CONST(4)=5.0
      CALL F4BAIRSTOW(3,4,24,A(1),B(1),CONST,100,PL,
     1  PR(1),RI(1),INAC)
      WRITE(4,445)INAC(1),INAC(2),(RI(I),I=1,3),(PR(I),I=1,3)
  445 FORMAT(' INAC(1)= ',I3,6X,' INAC(2)= ',I3/
     1  ' IMAGINARY PARTS OF TAU: ' ,3F8.3,/,' REAL PARTS',3F8.3)

C
      DO 447 I=1,3
  447 TAU(I)=PR(I)
      GO TO 550
  460 CONTINUE
C
C   CALCULATION OF AMPLITUDES
C
  550 IF(N.EQ.1)GO TO 555
      IF(N.EQ.2)GO TO 560
      IF(N.EQ.3)GO TO 565
      IF(N.EQ.4)GO TO 590
  555 A(1)=G(1)/TAU(1)
      GO TO 650
  560 PR(1)=TAU(1)*TAU(1)
      PR(2)=TAU(2)*TAU(2)
      A(2)=(PR(1)*G(1)-TAU(1)*G(2))/(PR(1)*TAU(2)-PR(2)*TAU
     1 (1))
      A(1)=(PR(2)*G(1)-TAU(2)*G(2))/(PR(1)*TAU(2)-
     1 PR(2)*TAU(1))
      GO TO 650
  565 CONTINUE
      GO TO 650
  590 CONTINUE
      GO TO 650
C
C   OUTPUT.
C
  650 WRITE(4,655)(TAU(I),I=1,N)
  655 FORMAT(' TAU VALUES    : ',4(2X,F12.3,3X)/)
      WRITE(4,660)(A(I),I=1,N)
  660 FORMAT(' AMPLITUDES    : ',4(2X,F12.3,3X)/)
      WRITE(4,665)(G(I),I=1,M)
  665 FORMAT(//' G(I):- ',/,4(2X,F12.3,3X,/))
      WRITE(4,670)(FMOM(I),I=1,M)
  670 FORMAT(//,' LAMP MOMENTS:- ',/,4(2X,F12.3,3X),/)
      WRITE(4,675)(DMOM(I),I=1,M)
  675 FORMAT(//,' DATA MOMENTS:- ',/,4(2X,F12.3,3X),/)
      IF(DEP.EQ.1.0)GO TO 690
      DIMENSION TAUB(4)
```

```
      DO 680 I=1,N
  680 TAUB(I)=TAU(I)/(1.0-DEP*TAU(I))
      WRITE(4,685)(TAUB(I),I=1,N)
  685 FORMAT(/,' UNDEPRESSED TAU VALUES:',4(F12.3,3X),//)
  690 CONTINUE
C
C
C
C   CUTOFF CORRECTION
C
      DIMENSION BETA(4)
      DO 710 J=1,N
      EXPT=EXP(TCH/TAU(J))
      EXPS=EXPT
      DO 700 I=1,NCH
      XINT(I)=F(I)*EXPS
  700 EXPS=EXPS*EXPT
      CALL SIMP(XINT,TCH,NCH,BETA(J))
  710 BETA(J)=BETA(J)*A(J)
C
      DIMENSION AIN(4,10)
      T=TCH*NCH
      DO 750 I=1,N
      EXPT=EXP(-T/TAU(I))
      AIN(I,1)=TAU(I)*EXPT
      EXPS=AIN(I,1)
      DO 750 J=2,M
      K=J-1
      TK=FLOAT(K)
      EXPS=T*EXPS
      AIN(I,J)=EXPS + TK*TAU(I)*AIN(I,K)
  750 CONTINUE
C
      DIMENSION DELTA(10)
      DO 800 I=1,M
      DELTA(I)=0.0
      DO 780 J=1,N
  780 DELTA(I)=DELTA(I)+BETA(J)*AIN(J,I)
  800 CONTINUE
C
      WRITE(4,810)(DELTA(I),I=1,M)
  810 FORMAT( ' CUTOFF CORRECTIONS:- ',4F12.5,/)
C
      AMAX=0.0
      DO 830 I=1,M
      G(I)=1 - OLDELTA(I)/DELTA(I)
  830 IF(G(I).GT.AMAX)AMAX=G(I)
      IF(AMAX.LT.0.005)GO TO 900
      DO 850 I=1,M
      DMOM(I)=DMOM(I) + DELTA(I) - OLDELTA(I)
  850 OLDELTA(I)=DELTA(I)
      GO TO 300
  900 CONTINUE
      STOP
```

```
C
C
      END
C
      SUBROUTINE SIMP(XINT,TCH,NCH,AREA)
C    XINT IS ARRAY OF NCH ORDINATES, AT INTERVAL TCH.
C     AREA IS COMPUTED AREA OUTPUT.
C       USES SIMPSONS RULE
        DIMENSION XINT(256)
        SUMA=0.0
        SUMB=0.0
        M=NCH-1
        DO 10 I=2,M,2
   10   SUMA=SUMA + XINT(I)
        M=NCH-2
        DO 15 I=3,M,2
   15   SUMB=SUMB + XINT(I)
        AREA=TCH*(XINT(1)+XINT(NCH)+4.*SUMA+2.*SUMB)/3.
        RETURN
        END
C
C
        FUNCTION FAC(I)
C     CALCULATES I FACTORIAL.
        N=1
        IF(I.LE.0)GO TO 20
        DO 10 J=1,I
   10   N=N*J
        FAC=FLOAT(N)
   20   IF(I.LT.0)GO TO 25
        FAC=1.0
        GO TO 30
   25   WRITE(4,27)
   27   FORMAT(/' ERROR: FACTORIAL OF NEGATIVE NUMBER ')
        STOP
   30   CONTINUE
        RETURN
        END
C
C
        FINISH
```

(Note – this program was originally intended for analyses up to 4 components, but was only implemented to 2)

ITERATIVE CONVOLUTION (LIFETIMES) PROGRAM

```
      LIST (LP)
      PROGRAM (CLFD)
      INPUT 1 = CR0
      INPUT 3 =TR1
      INPUT 4 = TR2
      OUTPUT 2 = LP0
      COMPRESS INTEGER AND LOGICAL
      COMPACT
      TRACE 0
      END
      TRACE 0
      READ FROM (CR)
      MASTER
      COMMON D(256),FLMP(256),NCHAN
      READ(1,50)NCHAN,BKGDAT,BKGLMP,GUESS
     1  ,FACT
   50 FORMAT(I0,4F0.0)
      READ(3,70)FST,(FLMP(I),I=1,NCHAN)
   70 FORMAT(256F0.0)
      READ(4,70)DST,(D(I),I=1,NCHAN)
      DO 160 I=1,NCHAN
      FLMP(I)=FLMP(I)-BKGLMP
      IF(FLMP(I).LE.0.0) FLMP(I)=0.0
      D(I)=D(I)-BKGDAT
      IF(D(I).LE.0.0) D(I)=0.0
  160 CONTINUE
C       NORMALISATION
      FMAX=FLMP(1)
      DMAX=D(1)
       DO 180 I=1,NCHAN
      IF(FLMP(I).GE.FMAX) FMAX=FLMP(I)
      IF(D(I).GE.DMAX) DMAX=D(I)
  180 CONTINUE
      DO 200 I=1,NCHAN
      FLMP(I)=FLMP(I)*100000./FMAX
      D(I)=D(I)*100000./DMAX
  200 CONTINUE
      CALL SEARCH(FACT,GUESS)
  900  CONTINUE
      STOP
      END
C
C
C
      SUBROUTINE SEARCH(FACT,TAUSRT)
      DIMENSION CONV(256),G(256),
     1 TSTOR(50),RSTOR(50),T(256)
      COMMON D(256),FLMP(256),NCHAN
      READ (1,40) JSIGNL
   40 FORMAT(I1)
10.34.55←
```

```
      NSTOP=50
   60 TAU=TAUSRT
      ICOUNT=0
      XINC=2.0
   80 KTEST=0
      GO TO 800
  100 RESID=0.0
      IF(JSIGNL.LE.0) GO TO 140
      DO 120 J=IMAX,NCHAN
      WEIGHT=D(J)
      IF(WEIGHT.LE.1.0) WEIGHT=1.0
  120 RESID=RESID + ((D(J) - CONV(J))**(2))*WEIGHT/10000.0
      GO TO 180
  140 DO 160 J=1,NCHAN
      WEIGHT=D(J)
      IF (WEIGHT.LE.1.0) WEIGHT=1.0
  160 RESID=RESID+((D(J)-CONV(J))**(2))*WEIGHT/10000.0
  180  RSTOR(ICOUNT)=RESID
  200 R1=RESID
  220 TAU=TAU + XINC
      IF(TAU.GT.50) GO TO 540
      DO 240 I=1,ICOUNT
      IF(TSTOR(I).EQ.TAU) GO TO 260
  240  CONTINUE
      GO TO 280
  260 RESID=RSTOR(I)
      GO TO 420
  280 KTEST=1
      GO TO 800
  300 RESID=0.0
      IF(JSIGNL.LE.0) GO TO 340
      DO 320 J=IMAX,NCHAN
      WEIGHT=D(J)
      IF(WEIGHT.LE.1.0) WEIGHT=1.0
  320 RESID=RESID+((D(J)-CONV(J))**(2))*WEIGHT/10000.0
      GO TO 400
  340  DO 380 I=1,NCHAN
      WEIGHT=D(I)
      IF(WEIGHT.LE.1.0) WEIGHT=1.0
  380 RESID=RESID+((D(I)-CONV(I))**(2))*WEIGHT/10000.0
  400 RSTOR(ICOUNT)=RESID
  420 R2=RESID
      WRITE(2,440)ICOUNT,TAU,R1,R2
  440 FORMAT(I4,2X,F8.4,2X,E12.5,2X,E12.5)
      IF(R2-R1) 460,480,480
  460 R1=R2
      GO TO 220
  480 IF(XINC.LE.0.005) GO TO 540
      TAU=TAU - 2.0*XINC
      IF(TAU.LE.0.0) TAU=0.5
10.39.44←
```

```
100         XINC=XINC/2.0
101         DO 500 I=1,ICOUNT
102         IF(TSTOR(I).EQ.TAU) GO TO 520
103     500 CONTINUE
104         GO TO 80
105     520 RESID=RSTOR(I)
106         GO TO 200
107     540 TAU=TAU - XINC
108         IF(JSIGNL.LE.0) GO TO 720
109         WRITE(2,560)
110     560 FORMAT(//,' FITTING FROM MAXIMUM'/)
111     580 WRITE(2,600) TAU,R1,ICOUNT
112     600 FORMAT(' BEST FIT TAU=', F8.4,/,
113        1   ' BEST FIT RESIDUAL= ', E12.5,/,
114        2    ' NUMBER OF CONVOLUTIONS PERFORMED= ', I4,/)
115 C
116         IF(JSIGNL-1) 980,980,700
117     700 JSIGNL=0
118         GO TO 60
119     720 WRITE(2,740)
120     740 FORMAT(//,' FITTING FROM FIRST CHANNEL'/)
121         GO TO 580
122 C
123     800 ICOUNT=ICOUNT +1
124         IF(ICOUNT.GE.NSTOP) GO TO 540
125         TSTOR(ICOUNT)=TAU
126         ZEX=EXP(-FACT/TAU)
127         G(1)=ZEX
128         DO 802 I=2,NCHAN
129         J=I-1
130         G(I)=G(J)*ZEX
131     802 CONTINUE
132         DO 803 J=3,NCHAN
133         I=0
134         SUM=0.0
135     804 I=I+1
136         M=J-I
137         SUM=SUM+G(I)*FLMP(M)
138         IF(M-1) 805,805,804
139     805 CONTINUE
140     803 CONV(J)=(FLMP(J)+2.*SUM)*FACT/2.
141         GO TO 806
142         SUM=FLMP(1) /ZEX
143         DO 880 J=2,NCHAN
144         XJ=J
145         SUM=SUM+FLMP(J-1)*EXP((XJ*FACT/TAU)
146        1   + FLMP(J)/G(J))
147         CONV(J)=(FACT/2.0)*SUM*G(J)
148     880 CONTINUE
149     806 CONTINUE
10.44.44←
```

```
    150         CONV(1) = 0.0
    151         CONV(2)=0.0
    152         CONMAX=CONV(3)
    153         DO 900 I=3,NCHAN
    154     900 CONMAX=AMAX1(CONMAX,CONV(I))
    155         DO 920 I=1,NCHAN
    156         CONV(I)=CONV(I)*100000./CONMAX
    157     920 CONTINUE
    158         DO 940 I=3,NCHAN
    159         IF(CONV(I).GE.99999.) GO TO 960
    160     940 CONTINUE
    161     960 IMAX=I
    162         IF(KTEST) 100,100,300
    163     980 CONTINUE
    164         RETURN
    165         END
    166         FINISH
10.46.40←
```

```
      LIST (LP)
      PROGRAM (CLFD)
      INPUT 1 = CR0
      INPUT 3 =TR1
      INPUT 4 = TR2                    LCALC
      OUTPUT 2 = LP0
      OUTPUT 6 = LP1
      COMPRESS INTEGER AND LOGICAL
      COMPACT
      TRACE 0
      END
      TRACE 0
      READ FROM (CR)
      MASTER
      DIMENSION DRF(256),FLMP(256)
      READ(1,50)NCHAN,BKGLMP,TRF,FACT
   50 FORMAT(I0,3F0.0)
      READ(3,70)FST,(DRF(I),I=1,NCHAN)
   70 FORMAT(256F0.0)
      IF(BKGLMP.EQ.0.0)GO TO 150
      DO 150 I=1,NCHAN
      DRF(I)=DRF(I) - BKGLMP
      IF(DRF(I).LT.0.0)DRF(I)=0.0
  150 CONTINUE
C       NORMALISATION
      RMAX=DRF(1)
      DO 180 I=1,NCHAN
      IF(DRF(I).GT.RMAX)RMAX=DRF(I)
  180 CONTINUE
      RMAX=10000./RMAX
      DO 200 I=1,NCHAN

      DRF(I)=DRF(I)*RMAX
  200 CONTINUE
C
C   CALCULATION OF THE LAMP FUNCTION FROM REF.
C   WAHL ET AL,REV SCI INSTR.,45,28,(1974)
C
      M=NCHAN-1
      CONST=TRF/(2.*FACT)
      RMAX=0.0
      DO 300 I=2,M
      J=I+1
      K=I-1
      FLMP(I)=DRF(I)+CONST*(DRF(J)-DRF(K))
      IF(FLMP(I).GT.RMAX)RMAX=FLMP(I)
  300 CONTINUE
      FLMP(1)=DRF(1)+(DRF(2)-DRF(1))*TRF/FACT
      J=NCHAN-1
      FLMP(NCHAN)=DRF(NCHAN)+(DRF(NCHAN)-DRF(J))*TRF/FACT
      IF(FLMP(1).GT.RMAX)RMAX=FLMP(1)
      IF(FLMP(NCHAN).GT.RMAX)RMAX=FLMP(NCHAN)
      RMAX=10000./RMAX
      DO 350 I=1,NCHAN
      FLMP(I)=FLMP(I)*RMAX
  350 CONTINUE
      WRITE(6,400)(FLMP(I),I=1,NCHAN)
  400 FORMAT(8F9.1)
      STOP
      END
      FINISH
```

BIBLIOGRAPHY

1. J.W. Longworth in 'Excited States of Proteins and Nucleic acids', ed. I. Weinryb and R.F. Steiner, Macmillan, London, (1971) p. 319
2. G. Weber and F.W.J. Teale, Biochem. J. 65, 476 (1957)
3. R.W. Cowgill, Biochem. Biophys. Acta, 133, 6 (1967)
4. A. White, Biochem. J. 71, 217 (1959)
5. J.W. Longworth and R.O. Rahn, Biochem. Biophys. Acta 147, 526 (1967)
6. R.F. Steiner and R. Kolinski, Biochemistry 7, 1014 (1968)
7. I. Weinryb and R.F. Steiner in ref. 1, p. 277
8. R.W. Cowgill, Biochem. Biophys. Acta 200, 18 (1970)
9. M. Nieto, H.R. Perkins, J.M. Frere, J.M. Ghuysen, Biochem. J., 135, 493 (1973)
10. T.Y. Tsong, J. Biol. Chem. 249, 1988 (1974)
11. H.J. Pownall and L.C. Smith, Biochemistry 13, 2594 (1974)
12. H.J. Pownall and L.C. Smith, Biochemistry 13, 2590 (1974)
13. A. Finazzi - Agro, C. Giovagnoli, L. Avigliano, G. Rotilio and B. Mondovi, Eur. J. Biochem. 34, 20 (1973)
14. A. Finazzi - Agro, G. Rotilio, L. Avigliano, P. Guerrieri, V. Boffi and B. Mondovi, Biochemistry 9, 2009 (1970)
15. W. Nieuwenhuizen, A. Oomens, G.H.de Haas, Eur. J. Biochem. 40, 9 (1973)
16. J.P.Abita, M. Lazdunskii, P.P.M. Bonsen, W.A. Pieterson, G.H. de Haas, Eur. J. Biochem. 30, 37 (1972)
17. H. Zimmermann and N. Joop, Z. fur Elektrochem., 65, 61 (1961)
18. J.R. Platt, J. Chem. Phys. 17, 484 (1949)

19. G. Weber, Biochem. J. 75, 335 (1960)

20. G. Weber in 'Light and Life', eds W.M. McElroy & B. Glass, The Johns Hopkins Press, Baltimore (1961), p 82.

21. E.H. Strickland, J. Horwitz and C. Billups, Biochemistry 9 4914 (1970)

22. E.H. Strickland, C. Billups Biopolymers 12, 1989 (1973)

23. Y. Yamamoto and J. Tanaka, Bull. Chem. Soc. Jap. 45,1362(1972)

24. I. Fischer-Hjalmers and M. Sundborn, Acta. Chem. Scand. 22, 607 (1968)

25. E.Pop, L. Klasinc and N. Trinjastic, Tetrahedron 28,3465(1972)

26. E.H. Strickland, C. Billups and E. Kay, Biochemistry 11, 3657 (1972)

27. W.E. Kurtin and P. S. Song, J. Amer. Chem. Soc.91, 4892(1969)

28. S.V. Konev, 'Fluorescence and Phosphorescence of Proteins and Nucleic acids', Plenum Press, NY (1967)

29. W.B. De Lauder and Ph. Wahl, Biochim. Biophys. Acta 243 153 (1971)

30. L.J. Andrews and L.S. Forster, Photochem. Photobiol. 19, 353 (1974)

31. N. Mataga, Y. Torihashi and K. Ezumi, Theoret. Chim. Acta 2, 158 (1964)

32. M.S. Walker, T.W. Bednar and R. Lumry, J. Chem. Phys 47, 1020 (1967)

33. J. Eisinger and G. Navon, J. Chem. Phys. 50, 2069 (1969)

34. C.M. Chopin and J.H. Wharton, Chem. Phys. Letts, 3,552(1969)

35. C.M. Chopin, Ph.D. Thesis, Louisiana State University 1972.

36. E. Vander Donckt, Bull. Soc. Chim. Belges, 78, 69 (1969)

37. B.L. Van Duuren, J. Org. Chem., 26, 2954 (1961)

38. R.F. Steiner and E.P. Kirby, J. Phys. Chem., 73, 4130 (1969)

39. J. Feitelson, Israel J. Chem. 8, 241 - 252 (1970)

40. R.P. Ambler and R. Brown, Biochem. J., 104, 784 (1967)

41. H.E. Fritz, J. Org. Chem. 28, 1384 (1963)

42. A.I. Vogel, 'Practical Organic Chemistry', Longmans, 1956

43. G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz and R. Terrell, J. Amer. Chem. Soc. 85, 207 (1963)

44. C.R. Hauser, F.W. Swamer and B.I. Ringler, J. Amer. Chem. Soc., 70,4023 (1948)

45. K.E. Pfitzner and J.G. Moffatt, J. Amer. Chem. Soc. 85, 3027 (1963)

46. N. Kornblum, W.J. Jones and G.J. Anderson, J. Amer. Chem. Soc' 81, 4113 (1959)

47. Ref. 42, p. 318

48. E.F. Pratt and L.W. Botimer J. Amer. Chem. Soc., 79 5248 (1957)

49. W.A. Jacobs and L.C. Craig, J. Biol. Chem. 113, 759 (1936)

50. W.J. Houlihan (ed) 'Indoles' Wiley (1972)

51. J.S. Lumsden, J. Chem. Soc. 87, 87 (1905)

52. A.J. Birch and H. Smith, Quart. Rev. (London) 12, 17 (1958)

53. L.N. Owen and P.N. Robins, J. Chem. Soc., 326 (1949)

54. E. Hardegger, Pl.A. Plattner and F. Blank, Helv. Chim. Acta 27, 793 (1944)

55. E.R.H. Jones and F. Sondheimer, J. Chem. Soc. 615 (1949)

56. J.P. Greenstein and M. Winitz, 'Chemistry of the Amino Acids' Wiley, London (1961)

57. J.B. Aldersley, G.N. Burkhardt, A.E. Gillam and N.C. Hindley, J. Chem. Soc. 10 (1940)

58. A.Z. Britten and G. Lockwood, J. Chem. Soc. Perk I 1824(1974)

59. J. Harley-Mason and E.H. Pavri, J. Chem. Soc. 2504 (1963)

60. R. Ratcliffe and R. Rodehorst, J. Org. Chem. 35, 4000(1970)

61. W.S. Trahanowsky, L.B. Young and G.L. Brown, J. Org. Chem. 32, 3865 (1967)

62. J.D. Albright and L. Goldman, J. Amer. Chem. Soc. 87, 4214 (1965)

63. H.C. Brown, C.P.Garg, and K.T. Liu, J. Org. Chem. 36 387 (1971)

64. W.J. Hickinbottom, 'Reactions of Organic Compounds', Longmans, London (1959)

65. H.O. House and H.W. Thompson, J. Org. Chem. 28, 365 (1963)

66. E. Stenhagen (ed) 'Atlas of Mass Spectral Data', Interscience, London, (1969) p. 1141

67. J.H. Bowie, Austral. J. Chem. 19, 1619 (1966)

68. A.Z. Britten, unpublished.

69. A. Brossi, A. Facella and S. Teitel, J. Med. Chem. 16 418 (1973)

70. K.T. Potts and J.E. Saxton, J. Chem. Soc. 2641 (1954)

71. 'Dictionary of Organic Compounds', Eyre & Spottiswoode, London (1965 and later supplements)

72. R.H. Cornforth and R. Robinson, J. Chem. Soc., 680 (1942)

73. B. Lacoume, G. Milcent, A. Olivier, Tetrahedron 28,667(1972)

74. W.H. Perkin, Jr, and S.G.P. Plant J. Chem. Soc. 123 3242 (1923)

75. S.G.P. Plant and D.M.L. Rippon, J. Chem. Soc. 1906(1928)

76. W.H. Perkin Jr. and S.G.P. Plant J. Chem. Soc. 2583(1928)

77. H.J. Teuber, D. Cornelius and E. Worbs, Tet. Letts.331 (1964)

78. M.W. Bullock and S.W. Fox, J. Amer. Chem. Soc. 73 5155 (1951)

79. J. Szmuszkovicz J. Amer. Chem. Soc. 79 2819 (1957)

80. V. du Vigneaud and R.R. Sealock, J. Biol. Chem. 96 511 (1932)

81. B. Witkop, J.B. Patrick and M. Rosenblum, J. Amer. Chem. Soc. 73, 2641 (1951)

82. C.U. Rogers and B.B. Corson J. Amer. Chem. Soc. 69 2910 (1947)

83. J.W. Bridges and R.T. Williams, Biochem. J. 107 225 (1968)

84. L.M. Rice, E. Hertz and M.E. Freed, J. Med. Chem. 7 313 (1964)

85. W.H. Perkin Jr., J. Chem. Soc. 85, 416 (1904)

86. T. Hoshino and K. Shimodaira, Ann. 520, 19 (1935)

87. O. Vogl and M. Pohm, Monatsh. 84, 1097 (1953); C.A. 49, 1560f (1955)

88. C. Doree and V.A. Petrow, J. Chem. Soc. 138 1391 (1935)

89. M.G. Lester, V. Petrow and O. Stephenson, Tetrahedron 21 1761 (1965)

90. D.J. Harvey and S.T. Reid, Tetrahedron 28, 2489 (1972)

91. Y. Ban and Y, Sato, Chem. Pharm. Bull. (Jap) 13 1073(1965)

92. D.J. Harvey, W.A. Laurie and R.I. Reid, Org. Mass. Spec. 5, 1183 (1971)

93. J.B. Birks 'Photophysics of Aromatic Molecules' Wiley - Interscience, London/NY 1970

94. R.W. Wagner, Ph.D. Thesis, Michigan State University 1971

95. M.S. Walker, T.W. Bednar, R. Lumry and F. Humphries, Photochem. Photobiol. 14 147 (1971)

96. J.E. Bernadin, Ph.D. Thesis, Oregon University 1970

97. F. Quadrifoglio and D.W. Urry, J. Amer. Chem. Soc. 90 2760 (1968)

98. B. Klabuhn, D. Spindler and H. Goetz, Spectrochim. Acta 29A 1283 (1973)

99. J.T. Davies and J.M. Vaughan, Astrophys. J. 137, 1302 (1963)

100. J. Aitchison and J.A.V. Brown, 'The Lognormal Distribution' Cambridge University Press 1957

101. D.B. Siano and D.E. Metzler, J. Chem. Phys. 51, 1856 (1969)

102. R.B. Johnson and D.E. Metzler, Methods in Enzymology 18A 433 (1970)

103. J. Pitha and R.N. Jones, Can. J. Chem. 44, 3031 (1966)

104. J. Meiron, J. Opt. Soc. Amer. 55, 1105 (1965)

105. D.E. Metzler, C. Haras, I.Y. Young, D. Siano and J.A. Thomson, Biochem. Biophys. Res. Comm. 46, 1588 (1972)

106. C. Aussems, S. Jaspers, G. Leroy,and F. Van Remoortere, Bull. Soc. Chim. Belges. 78, 479 (1969)

107. P. Carles and A. Julg, J. Chim. Physique 65, 2030 (1968)

108. R. Andrisano and T. Vitali, Gazz. Chim. Ital. 87,949(1957)

109. W. West in 'Chemical Applications of Spectroscopy', vol IX pt.1, ed A. Weissberger (1968)

110. D.E. Metzler, C.M. Harris, R.J. Johnson, D.B. Siano and J.A. Thomson, Biochemistry 12, 5377 (1973)

111. E.G. McRae, J. Phys. Chem. 61, 562 (1957)

112. N. Mataga and T. Kubota, 'Molecular Interactions and Electronic Spectra', Dekker, NY (1970) chap. 8

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

114. E. Lippert, W. Luder and H. Boos Adv. Mol. Spectrosc. 1, 443 (1962)

115. J.W. Longworth, Photochem. Photobiol. 7 587 (1968)

116. The C.R.C. Handbook of Physics and Chemistry. C.R.C.

117. C. Cazeau-Dubroca, F. Dupuy, M. Martineaud and A.L. Campillo Chem. Phys. Letts. 23, 397 (1973)

118. M.G. Reinecke, H.W. Johnson Jr, and J.F. Sebastian, J. Amer. Chem. Soc. 91, 3817 (1969)

119. A. Szent-Gyorgyi, Proc. Nat. Acad. Sci. 46, 1334 (1960)

120. R. Foster and C.A. Fyfe, J. Chem. Soc.(B) 926 (1966)

121. J.Green and J.P. Malrieu, Proc. Nat. Acad. Sci. 54,659(1965)

122. B. Stevens and M.I. Ban, Trans. Far. Soc. 60, 1515 (1964)

123. U Liddel and E.D. Becker, Spectrochim. Acta. 10, 70 (1957)

124. D.L. Dexter, J. Chem. Phys. 21, 836 (1953)

125. A.A. Lamola in 'Energy Transfer and Organic Photochemistry' (Technique of Org. Chem., Vol. XIV, ed Weissberger) Interscience (1969) p. 26

126. M. O'Sullivan and A.C. Testa, J. Phys. Chem. 77 1830 (1973)

127. B.J. Tabner, J.R. Yandle 'Reactions of Molecules at Electrodes' ed Hush (1971) p. 298

128. B. Case et. al. J. Electroanalyt. Chem. 10, 360 (1965)

129. H.R. Mahler and E.H. Cordes, 'Biological Chemistry ' Harper & Row (1966) p. 13

130. I. Weinryb and R.F. Steiner, Biochemistry 7 2488 (1968)

131. Ref. 93, p 130

132. J. Tournon and M.A. El-Bayoumi, J. Chem. Phys. 56 5128(1972)

133. N. Christodouleas and S.P. McGlynn, J. Chem. Phys. 40 166 (1964)

134. A.A. Lamola and G.S. Hammond, J. Chem.Phys. 43, 2129 (1965)

135. S. Searles, M. Tamres and G.M. Barrow, J. Amer. Chem. Soc., 75, 71 (1953)

136. M.L. Josien, N. Fuson, J.M. Lebas and T.M. Gregory, J. Chem. Phys. 21, 331 (1953)

137. T.R. Hopkins and R. Lumry, Photochem. Photobiol. 15 555 (1972)

138. J.N. Demas and G.A. Crosby, J. Phys. Chem. 75, 991 (1971)

139. C.A. Parker and W.T. Rees, Analyst 85, 587 (1960)

140. V.A. Mode and D.H. Sisson, Anal. Chem. 46, 200 (1974)

141. W.H. Melhuish, J. Res. Nat. Bur. Stand. 76A, 547 (1972)

142. W.R. Ware in 'Creation and Detection of the Excited State' Vol. IA, ed A.A. Lamola. Dekker, NY. (1971)

143. C. Lewis, W.R. Ware, L.J. Doemeny and T.L. Nemzek, Rev. Sci. Instr. 44, 107 (1973)

144. I. Isenberg, R.D. Dyson and R. Hanson, Biophys. J. 13 1090 (1973)

145. I. Isenberg, J. Chem. Phys. 59, 5696 (1973)

146. I. Isenberg, J. Chem. Phys. 59, 5708 (1973)

147. Ph. Wahl, J.C. Auchet and B. Donzel, Rev. Sci. Instr. 45 28 (1974)

148. W.R. Ware and C. Lewis, J. Chem. Phys. 57, 3546 (1972)

149. G.S. Hammond, P.A. Leermakers and N.J. Turro, J. Amer. Chem. Soc. 83, 2396 (1961)

150. G.S. Hammond, N.J. Turro and P.A. Leermakers, J. Phys. Chem. 66, 1144 (1962)

151. G.S. Hammond et. al. J. Amer. Chem. Soc. 86, 3197 (1964)

152, C.G. Hatchard and C.A. Parker, Proc. Roy. Soc. A 235, 518 (1956)

153. R.N. Jones, R. Venkataragharan and J.W. Hopkins, Spectrochimica acta 23A 925 (1967)